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CATTLE PLAGUE VACCINE.
STUDIES ON GLYCERINISED SPLEEN PULP.

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SINCE the appearance of the first publication by Kakizaki (1918) on the value of glycerinised spleen pulp of infected cattle as a vaccine for cattle plague, a considerable literature has accumulated on the similar use of a number of tissues treated in various ways. It is, however, interesting to note that following a second paper by Kakizaki (1925), in which material advances in the study of glycerinised virus were recorded, experiments on cattle plague vaccine took an entirely new turn.

Observations—unfortunately leading to no great practical application—by Vallée, Carré and Rinjard (1925, 1926) on the use of formalinised foot-and-mouth disease virus as a vaccine, led Curasson and Delpy (1926) to attempt the adaptation of their technique to cattle plague vaccination. The success that attended their efforts, reinforced no doubt by the equally striking success of a vaccine subsequently developed along identical lines for canine distemper, led to widespread study of formalinised tissue vaccines and almost complete neglect of the original glycerinised vaccine of Kakizaki.

Recent years have seen the introduction of numerous modifications in the technique of preparing tissue vaccines, both as to the tissues utilised and the chemical agents incorporated with them. Many of these modifications have proved useful but, among the agents used for treating virulent tissues, the two original compounds, namely glycerol and formaldehyde, still retain pride of place, and of these the latter enjoys the greater popularity.

No work of any kind was conducted on cattle plague in the Sudan until 1928, leaving one, in so far as the technique of vaccine preparation was concerned, in the fortunate position of a disinterested spectator. When, however, the production of antiserum

was undertaken in that year it became possible to institute studies on vaccines.

In contemplating the preparation of cattle plague vaccine in the Sudan the peculiar local conditions had always to be borne in mind. Of these, the most essential is the seasonal nature of routine serum production, which is determined by prevailing climatic conditions. Serum preparation on a large scale can only be economically carried out in the Southern Sudan, since it is only in the south that permanent grazing is available. Heavy seasonal rains and floods, however, limit operations to little more than half the year—roughly December to June inclusive, so that in order to supply the country's annual requirements a considerable reserve must be accumulated by the middle of the year. Thus, since vaccine could only be economically prepared simultaneously with serum, one had to aim at a vaccine capable of being stored for several months.

As will be seen in the section dealing with the storage capacity of the vaccine, the literature was at that time, and in fact still is, lacking in precise information on this, to the Sudan, most essential factor. Nevertheless, a study of the existing literature led to the fairly certain conclusion that, with the necessity for storage as a major point at issue, the most promising type of tissue vaccine to study would be that prepared with glycerine.

In any case, formalinised tissue vaccines were not suffering from lack of study, and in instituting experimental work on the glycerinised type an advantage might accrue, in that the essential properties of formalinised vaccines would probably be determined with considerable precision in advance of one's own results being obtained. It was, in fact, expected that as soon as any precise result was established it would be open to comparison with its counterpart in the formalinised range in more or less absolute terms. In the event this hope has not been realised, and although the essential properties of glycerinised vaccine have been reduced to more or less absolute terms it is still not possible to draw close comparison with the formalinised product.

The experiments have been in progress for six or seven years, but, owing to preoccupation with other duties, long breaks in continuity will be apparent. Nevertheless, by arranging the records in convenient sections, it is hoped that the spasmodic and opportunistic manner in which the work was done will not unduly interfere with the intelligibility of one's observations. Since the reasons for each series of experiments will be discussed in their appropriate sections, it will be convenient to proceed immediately to a description of the work.

Preparation of the Vaccine.

A study of the literature on formalinised tissue vaccine shows that there has been wide variation in the technique of preparation.

Different concentrations of formaldehyde, in varying proportions to the tissue, and a range of tissues, sometimes separately and sometimes mixed, have been used. Such wide superficial ranging may be justified in the early stages of any study, but inherent in the process is a danger of its leading to confusion in that the results of different workers, and even sometimes of the same worker, are not comparable. This danger in the case of cattle plague vaccine has probably proved more potential than real, but when instituting one's own work it was decided to eliminate it by designing one's basic experiments as a study of the properties of the vaccine virus in a single tissue prepared according to a single technique. The study of other body tissues, and possible modifications in the technique of preparation, were relegated to a future which, it was hoped, would have a fully understood " standard " vaccine as a basis for comparison.

Kakizaki (1918) carried out his original observations on glycerinised spleen tissue and later (1925) defined his " glycerine water " as being two parts of 60 per cent. aqueous glycerine added to one part of spleen pulp. The more fundamental of his later experiments were also carried out with this mixture. The only spleen vaccine used in all experiments recorded in the present paper has therefore been prepared according to Kakizaki's original technique, namely to remove spleens from cattle plague patients at the height of an acute febrile reaction, mince, weigh, and add double the weight of 60 per cent. aqueous glycerine.

Controls.

Before detailing the actual experiments, a note on the nature and susceptibility of the cattle employed in them is indicated. These have been young native bulls, about two years of age, and weighing about 300 to 350 lb. A plentiful and relatively cheap supply of these is available in the vicinity of Khartoum, and, in virtue of their being drawn from scattered small villages, they are more uniformly susceptible to cattle plague than would be similar cattle obtained from large nomadic herds.

The following is a statement of susceptibility in 250 consecutive " controls," etc., cattle of this type infected in the laboratory :—

A.	*Susceptible.*			
1.	Died	101	= 40·4 per cent.	} 66·4 per cent.
2.	Slaughtered for virus or vaccine	65	= 26·0 ,,	
3.	Developed cattle plague and recovered	51	= 20·4 ,,	
		217	86·8 ,,	
B.	*Immune*	33	= 13·2 ,,	
		250	= 100·0 ,,	

Examination of random sectors of 50 consecutive cattle in this range affords almost exactly the same proportionate results. Controls have always been included in every infection experiment, but only sufficient have been used to prove the virulence of the virus used on the particular occasion. Since they have nearly always been much fewer than the definite animals, it is to the foregoing table that reference should be made in assessing the outcome.

It will be seen that susceptible cattle classed as " died " have been tentatively bracketed with those " slaughtered for virus or vaccine." This is largely, if not entirely, justified because the latter were specially selected as having developed the most acute attack of the disease. For rough practical calculation it may be reckoned that in the type of cattle used in these experiments there is a morbidity rate of about 85 per cent. and a mortality rate of about 65 per cent.

Safety Interval.

Among the contrasting features of formalinised and glycerinised vaccines, none is more noteworthy than the period elapsing between the preparation of vaccine from virulent tissue and its complete loss of virulence.

With the formalinised product there has from the outset been such general agreement that " inactivation " is very rapid, that few workers have troubled to study the question. For example, Curasson and Delpy (1926), using a final concentration of 0·2 per cent. formaldehyde, postulate 40 hours at " room temperature " (? 30° C.); no protocols are given to show that this is truly the minimum safety interval. Daubney (1928), using 0·1 per cent. formaldehyde and storing at ±5° C., demands 72 hours. Here again no proof is given that this period is a minimum, but with a 0·05 per cent. concentration he finds that the product still retains some virulence at the end of 92 hours. Another record of treatment with a lower concentration of formaldehyde than 0·1 per cent. is by Cooper (1930), who with 0·066 per cent. found the virulence not completely destroyed in four days at room temperature. Hall (1930), by means of serial tests of a sample of vaccine stored at room temperature (? 30° C.) with a formaldehyde concentration of 0·2 per cent., found that virulence persisted for at least six but not for 18 hours.

Formaldehyde, therefore, subject perhaps to a minimum concentration of about 0·1 per cent., renders cattle plague material avirulent in a few hours. The temperature of conservation, moreover, appears to be a feature of no material significance. It may, in passing, be noted that the same observations are applicable to toluol, *vide* Walker (1928, 1930), to chloroform, *vide* Kelser (1927), and probably also to phenol, *vide* Evans and Cornell (1929).

With the glycerinised product the case is quite otherwise. Kakizaki's first (1918) paper shows that temperature at any rate has considerable influence on the rate at which virulence disappears. A sample of glycerinised spleen stored at room temperature ranging from 15° to 20° C. was avirulent in 60, and probably in 45 days (protocols rather vague), while another sample stored at a temperature of −4° to 14° C. remained virulent for 60 but not for 90 days. Later (1925) a careful study of his protocols, in which the room temperature is recorded, shows that a sample stored at −2° to 13° C. was virulent after 60 but not after 124 days. In a third publication (1927) he shows that " heating " glycerinised spleen pulp destroys the virulence more rapidly without simultaneously destroying the antigenic value. " Heating " in this case meant placing in an incubator at 37° C. for a week.

One objection that has been raised to the general use of glycerinised vaccine is the long and variable period that must elapse before it is safe for use. A careful study of Kakizaki's protocols, however, indicated that with proper management of temperature the period need not necessarily be long—perhaps not significantly longer than when formalin is used. The variability, moreover, would only be a drawback if it were uncontrollable, and there seemed every indication that it could be fairly easily controlled, and thus turned into an advantage. The advantage would arise out of a possible relationship between the rate of loss of virulence and the rate of loss of antigenic value in glycerine medium.

Having conceived temperature to be one's critical factor, it did not seem necessary, either in these estimations of the safety interval or in other contemplated experiments, to work at more than two standard temperatures, *viz.*: 0° C. and 37° C. These temperatures are precise, and they are readily available in any bacteriological laboratory, thus enabling one to dispense with the entirely unsatisfactory " room temperature." With a view to subsequent experiments on storage and distribution of vaccine, 0° C. would represent an attainable storage temperature in a centralised laboratory in Khartoum, while 37° C. would be at least as high as temperatures at which vaccine would be stored in out-stations.

The following experiments were carried out to determine the safety interval.

16.12.28. *Preliminary experiment on loss of virulence at* 37° C.

Vaccine was prepared from a single spleen and stored in an incubator at 37° C. for ten days. Two young bulls were then given 5 c.c. each. Neither developed any symptoms whatsoever, and when tested 20 days later for immunity they again developed no symptoms, whereas two controls died of cattle plague.

30.11.30. *Preliminary experiment on loss of virulence at 0° C.*

Vaccine was made from three spleens and stored in a mixture of ice and water, in the hope that the temperature would remain at about 0° C. This hope was not realised, since as much ice as could be accommodated consistently melted overnight and the temperature rose by morning to about 20° C. Nevertheless, the temperature throughout most of the period was much lower than this.

When the vaccine had been stored for one month, four bulls were injected subcutaneously, two each with 5 c.c. and 25 c.c. The results are shown in Table I.

TABLE I.

INOCULATION OF CATTLE WITH GLYCERINISED SPLEEN STORED IN COLD FOR ONE MONTH.

Bull No.	*Dose.*	*Symptoms.*	*Result.*
607	5 c.c.	Mild fever, 13th to 17th days. Slight vesiculation of mouth, 15th to 18th days	Recovered
611	5 c.c.	Fever, 8th to 13th days. Slight vesiculation of mouth, 11th to 14th days	,,
614	25 c.c.	? ? Thermal reaction	,,
616	25 c.c.	Severe cattle plague	Died

Twenty-nine days later the three survivors, together with two controls, were given virulent blood. Both controls developed acute cattle plague and were " bled out " for virus, while none of three vaccinated cattle developed any symptoms whatsoever.

These two small preliminary experiments show without any doubt that virulence is conserved much longer at low than at high temperatures. Little else is proved, but certain valuable indications can be deduced. For example, the complete avirulence of the material in the first experiment and the solid immunity of the test cattle shortly afterwards indicate that absolute safety and high antigenic value are not incompatible. Regarding dosage and immunity interval, there are at least indications that active immunity of satisfactory solidity can be produced after a reasonable interval with doses of economic size.

Having determined one's preliminary " bracket," whereby vaccine stored at 37° C. was safe in less than ten days, while that stored at a little over 0° C. was not safe at the end of a month, a single experiment of greater precision was designed.

Experiment of 22.2.31.

Vaccine was prepared from the spleens of two young bulls and divided into two equal parts, one of which was stored at 37° C. and the other in a bath of ice water. The former was tested as to virulence at doses of 5 c.c., 20 c.c. and 50 c.c. at intervals of three,

six and nine days, while the latter was similarly tested at fortnightly intervals until it was found to be completely avirulent. As in the preliminary experiment, it was not found possible to keep the " cold " sample quite at 0° C., but the temperature rarely rose above 10° C. and that only for very short periods. The results of this experiment are shown in Table II.

TABLE II.

ESTIMATION OF SAFETY INTERVAL IN PORTIONS OF A SINGLE MIXED SAMPLE OF VACCINE STORED AT 37° C. AND AT A FEW DEGREES OVER 0° C.

Temp. of Storage.	*Period of Storage.*	*Dose.*	*Bull No.*	*Symptoms.*	*Result.*
37° C.	3 days	5 c.c.	662	No symptoms in any individual	All survived
		20 c.c.	663	„ „	„
		50 c.c.	665	„ „	„
	6 days	5 c.c.	666	No symptoms in any individual	All survived
		20 c.c.	668	„ „	„
		50 c.c.	671	„ „	„
	9 days	5 c.c.	669	No symptoms	All survived
		20 c.c.	672	„	„
		50 c.c.	674	Swelling at sight of injection, 2nd to 6th days, max. 3 × 4 in.	„
A few degrees over 0° C.	2 weeks	5 c.c.	667	Severe cattle plague	Died
		20 c.c.	670	„ „	„
		50 c.c.	673	„ „	„
	4 weeks	5 c.c.	679	No symptoms	Survived
		20 c.c.	681	No fever, but mild stomatitis, 10th and 11th days	„
		50 c.c.	688	Severe cattle plague to the point of violent dysentery	„
	6 weeks	5 c.c.	678	No symptoms	Survived
		20 c.c.	683	„	„
		50 c.c.	689	Swelling at site of injection, 2nd to 4th days, max. 3 × 3 in.	„

In regard to the main point at issue, namely the effect of temperature on safety, the data in Table II confirm the results of the preliminary experiments; the virulence of a single mixed vaccine persisted much longer at the lower temperature. Storage at 37° C. appears completely to destroy virulence within three days, while, as to storage at low temperature, the slightly lower degree attained in this instance was accompanied by the retention of virulence for a longer period than in the preliminary experiment, thus more strongly confirming that conservation of virulence is a function of temperature.

The question of dosage also calls for comment. It will be seen, both in this and in the earlier experiment, that with the cold storage samples in which virulence had partially but not completely disappeared, the fatally affected cattle were those which

received the largest dose. If a purely quantitative destruction of virus is at issue this is what might be expected. It cannot, however, be said that the observation of half-a-dozen cattle, of which two died, is sufficient on which to base a claim that such destruction is actually what occurs. The point is raised at this stage, not for discussion, but in order that it may be mentally registered for reconsideration when loss of antigenic value under varying conditions of temperature is discussed.

All survivors of the safety tests were ultimately lumped together and tested for immunity. Owing to their having been given vaccine at different times, the single immunity test was carried out at intervals varying from 18 to 56 days after the date of vaccination. The controls developed cattle plague, while not one of the vaccinated cattle developed any symptoms whatsoever. While this immunity test was merely normal routine, the observation that solid immunity was developed in not more than 18 days was a useful guide in subsequent immunity tests.

The foregoing experiments demonstrated beyond question the influence of temperature on conservation of virulence, but, with the installation of electric refrigerators in which the temperature could be properly controlled, it was thought advisable to carry out one more definitive experiment. The whole experiment had wider issues than the determination of the safety interval, but portions of its bearing on this factor must be recorded at this point.

Experiment of 18.1.32.

Vaccine was made from the spleens of three bulls, the final product being divided into two equal parts and stored at 37° C. and 0° C. respectively. On this occasion the sample in cold storage was actually maintained at a temperature varying only by a fraction of a degree from 0° C. For the main purpose of the experiment a large number of cattle had to be given vaccine as soon as it was safe. It was already known that storage at 37° C. for three days rendered it safe, and there seemed to be no object in finding out whether it would be safe in a rather shorter period. So far as the sample stored at 37° C. was concerned, to vaccinate cattle after three days' storage was merely a large scale confirmation of the preceding experiment, but in the case of the samples stored at 0° C. the safety interval had to be re-determined, by periodic testing for virulence on small groups of cattle, before vaccinating the cattle destined for the main experiment. The main group would then serve as a large scale confirmation of one's determination. The results of the experiment, in so far as the safety intervals are concerned, are given in Table III.

Comparison of the data in this table with those of the earlier experiments confirms that the lower the temperature of conservation, the longer virulence (or for vaccinating purposes, danger) is conserved. There is, of course, no point in working out the

TABLE III.

SAFETY INTERVAL, SINGLE MIXED SAMPLE OF VACCINE, PORTIONS STORED AT 37° C. AND 0° C. RESPECTIVELY.

Temp. of Storage.	*Period of Storage.*	*Dose.*	*Bull No.*	*Symptoms.*	*Result.*
37° C.	3 days	5 c.c.	8 bulls	No symptoms	Survived
		20 c.c.	8 ,,	,,	,,
		50 c.c.	8 ,,	Slight swellings at site of inoculation for a few days	,,
0° C.	6 weeks	50 c.c.	820	No symptoms (? naturally immune)	Survived
			821	Severe cattle plague	Died
	8 weeks	25 c.c.	848	Severe cattle plague	Died
			854	No symptoms (? naturally immune)	Survived
			855	Severe cattle plague	Died
	10 weeks	25 c.c.	894	Severe cattle plague	Died
			895	,, ,,	,,
			897	,, ,,	,,
	14 weeks	25 c.c.	882	No symptoms	Survived
			887	,,	,,
			891	,,	,,
	16 weeks	5 c.c.	8 bulls	No symptoms	Survived
		20 c.c.	8 ,,	,,	,,
		50 c.c.	8 ,,	,,	,,

interval for a complete range of temperatures, but at the two temperatures selected for study the period can be stated in sufficiently absolute terms for practical purposes, *viz.*, on storage at 37° C. the vaccine is safe for use within three days, while on storage at 0° C. it is not safe for some period in the region of three months. It is thus evident that a glycerinised vaccine can be made safe in three days. In practice this may not always be necessary. Nor may it always be even desirable, for if it is later found that antigenic value, as well as virulence, is better conserved at low temperatures, then unless the vaccine is needed for immediate use it will be better to store in the cold from the moment of preparation. The important fact, however, is that the danger/safety factor can be controlled at will, and that any sample of vaccine can rapidly be made safe.

These observations are approximately what might have been expected from a study of Kakizaki's original ones, and, as their bearing will be more easily appreciated by consulting the later sections than by discussion at this point, it is not now proposed to discuss them further.

A final mention of the effect of cold storage on conservation of virulence may, however, be made, as this has a distinct bearing on one's future experiments. Jacotot (1932) found that cold storage alone, *i.e.*, in the absence of glycerine treatment, permitted the conservation of virulence for seven months at 0° C.,

but that as soon as virulence disappeared antigenic value was also lost. This is certainly not the case with glycerinised tissue, as will be plainly seen in subsequent sections.

Before leaving the section on safety, it is as well to mention the irritation caused to vaccinated cattle by the glycerine content of the vaccine. In these early experiments some irritation and local swelling was noticed, the effect being in proportion to the dose. Nevertheless, throughout the whole of the experiments recorded in this paper only one bull (50 c.c. dose) developed a local swelling that could be regarded as seriously objectionable. In this case an abscess developed, but it is not necessarily admitted that it was caused by the glycerine. With the doses ultimately found sufficient for general use the irritation is very mild and transient.

"Fresh" Vaccine: Dosage and Immunity Interval.

Some explanation of the title of this section may be considered necessary. The primary object of all one's experiments is to determine a dose of vaccine suitable for general field use, and to effect this determination with full cognisance of the properties and limitations of one's product.

In the first place it seems that one should not determine a dose without some clear idea of what this dose should accomplish. Ultimately, of course, it should produce immunity in vaccinated cattle, but, since it is not improbable that the size of the dose may influence the rate of immunity production, it is highly desirable to study the dosage and the immunity interval concurrently. This procedure has been adopted throughout the studies here recorded.

Next, regarding the factor of freshness. Any sample of vaccine will be to some extent aged before it is actually injected, even in circumstances where vaccine production is in progress throughout the year. In the Sudan, as already explained, if the most economical method of preparation is to be practised, vaccine will have to be stored for several months. Cold storage may permit of the holding of centralised stocks over relatively long periods, but with the long distances, high temperatures, and indifferent transport facilities of the Sudan, further ageing influences will come into force before the vaccine is actually used. It is therefore obvious that the factor of freshness may have considerable influence on the dose-immunity-interval complex, but such influence cannot be appreciated until preliminary determinations have been made with freshest possible vaccine. It is on this account that a special section is allotted to consideration of such vaccines.

For the purposes of this section "fresh" vaccine is defined as approximately the youngest vaccine that is safe for use, *viz.*, glycerinised spleen stored for not much more than three days at

37° C. or three months at 0° C. Samples that had become safe on storage at both these temperatures were studied until it was found that their properties were identical.

No great care was indicated in selecting the doses for this preliminary investigation, since it was always possible that when stored vaccine had been similarly studied they might have to be altered. The only essential was to confine oneself to consideration of a *single* dose technique, since under Sudan conditions multiple inoculations or treatments of any kind are quite impracticable. It was thus decided to select " small," " medium " and " large " doses in the light of existing indications.

The literature was not as useful as might have been hoped in helping one to select a range of doses. Kakizaki (1918) appears originally not to have given the matter direct attention, but from his protocols it may be elicited that with a vaccine prepared from an unstated number of spleens, and stored at winter room temperature for 90 days, two cattle given *single* doses containing 0·66 gm. spleen developed " slight symptoms " of cattle plague in an immunity test carried out at an unstated interval after vaccination. In spite of this promising outcome from a small single dose, he follows up with observations on large multiple ones and finally concludes that two doses of 20 c.c. and 30 c.c. (6·6 gm. and 10 gm. spleen) are necessary. In a later paper (1925) he specifically studies a " one inoculation system " but apparently with the object of determining the immunity interval only. With doses of 50 c.c. and 80 c.c. (*ca.* 16 gm. and 26 gm. spleen) he finds that immunity develops in seven but not in four days. Kakizaki *et al.* (1927) record a large number of experiments, from which it is not easy to extract precise information. The vaccine employed, however, was " fresh," and single doses of 1·5 gm. to 5 gm. per 100 kg. body weight—varying apparently in different samples—produced immunity within ten days; shorter intervals than ten days were not tried. Later Japanese work confirms these observations, but there appears to be a disinclination to test immunity within ten or twelve days of vaccination. It may be mentioned that as a rule single beasts only are used by the Japanese workers in testing each dose of a sample, and controls are rarely included. In principle this is objectionable, but it cannot be denied that viewed as a whole their results are fairly consistent.

No other workers have studied plain glycerinised tissues, but some have prepared glycerinised vaccines to which some potent disinfectant such as formaldehyde, phenol or toluol has been added. When the principle involved is considered it would perhaps be better to say that they have added glycerine to formalinised, etc., vaccines, in which case the reason for adding the glycerine is not quite clear. Nevertheless, observations made on them may be of some comparative value and may be briefly reviewed.

Cornell and Evans (1931) concluded that 3 gm. of glycerinised spleen, either formalinised or phenolised, produce a serviceable immunity in one week; large doses, moreover, seemed to be no more effective than minimal ones. Their protocols, apart from the use of single beasts only, do not entirely support the first conclusion, since one beast, which had received 3 gm. of vaccine one week old, died in the immunity test seven days after vaccination. Nevertheless, a dose of 2 gm. glycerinised formalinised spleen appears subsequently to have been adopted as the standard dosage in Tanganyika Territory. Jacotot and Colson (1934) found that in the case of toluolised glycerinised spleen 1 to 2 gm. would prevent diarrhœa, but permit fever and catarrh to develop, in immunity tests carried out 15 days after vaccination. Before leaving the subject of vaccines made with disinfectant and glycerine it is interesting to note that Kakizaki *et al.* (1928) concluded that plain glycerinised spleen was superior to the same material with phenol or toluol added.

As regards formalinised spleen, opinions on dosage are extremely variable. Curasson and Delpy (1926) in their first publication found that 5 gm. spleen produced fairly good immunity within 15 days; beasts receiving 1 and 2 gm. developed cattle plague, while those receiving 5 gm. developed fever only on testing after this interval. However, in these experiments the vaccine was made from one spleen, and only one beast was used in each section of the test, so that the results cannot be accepted as representative. Later (1929), with pooled spleens, they found that 5 gm. immunised while 2 gm. did not. Moreover, 5 gm. did not produce immunity in three, seven or eight days but did so in ten days. The use of mixed tissue vaccines makes it impossible to consider later work in French West Africa.

Daubney (1928) found that 1 gm. fresh formalinised spleen would produce immunity in more than four but less than eleven days, the intervening interval not being studied. It is seen from the protocols of his immunity tests, however, that considerable reactions occurred in cattle which had received much larger doses than 1 gm. The routine dose in Kenya has subsequently been standardised at 3 gm.

Evans and Cornell (1929) provide much the same information. They say that 1 gm. spleen is sufficient to prevent the development of anything more than a febrile reaction in immunity tests 13 days or more later. In their protocols, however, one beast is recorded which received 6 gm. and developed clinical cattle plague when tested at a longer interval than 13 days. The routine dose in Tanganyika Territory has since been fixed at 2 gm.

Hall (1930), using doses of 8 gm., found that on exposure to contact infection vaccinated cattle acquired the disease if exposed during the first nine days. Later (1931) he found that up to 13 days after vaccination severe reactions or even fatal infection

developed in cattle exposed by contact; the dose in this case was 5 gm. spleeen, thus implying, although it is not stated, that with a smaller dose a longer immunity interval may be the rule.

Jacotot (1929), using 15 to 20 gm. of tissue, found immunity " pronounced " after eight days and complete after 15 days.

Walker (1931), using a range of doses, concludes that the rate of immunity production is proportional to the dose; large doses producing immunity in five days, while smaller ones require eight days. Few cattle are used in his experiment, however.

It is not easy to summarise the foregoing evidence so as to draw any very precise conclusions. There is no solid evidence, in spite of the conclusions of some workers, either for or against the size of the dose, whether of glycerinised or of " disinfected " vaccine, having any influence on the rate of immunity production; nor is there any evidence that the freshness or otherwise of the vaccine has any influence on the dosage or immunity interval. Admitting these fundamental failings, however, there are some valuable indications. In the case of formalinised vaccine, with which this paper is only indirectly concerned, it seems (*a*) that 1 gm./100 kg. is definitely the minimum dose, even with the best samples of the freshest vaccine, and (*b*) that whatever the dose, a serviceable (not necessarily solid) immunity will not develop in less than a week, the probabilities being in favour of an interval of at least ten days. In the case of plain glycerinised spleen (*a*) there is no evidence worth having on the size of the minimum dose, but the product seems to be at least as potent as its formalinised counterpart, and (*b*) such little evidence as is available as to immunity interval has a slight bias in favour of this product as compared with formalinised vaccine.

It is now convenient to describe one's own observations on " fresh " glycerinised spleen.

Experiment of 18.1.32.

A pooled vaccine was made from the spleens of three young bulls. Half of this was placed in an incubator at 37° C. and the other half in a refrigerator at 0° C. The former was tested after three days' incubation and the latter after four months' storage. (These samples have already been mentioned, *see* Table III.)

The form of test to which each sample was submitted was to give a range of doses of 1 gm., 4 gm. and 10 gm. per 100 kg. to 24 cattle,* eight at each dose, and to test two of each group as to immunity at intervals of three, eight and 13 days. The controls to one section of the test were thus just fit to provide virus for the next section, and so on. It was originally intended to carry out a further test on the eighteenth day, and sufficient cattle were

*The cattle used were two-year-old Arabs selected to be of fairly even type, and weighing 300 to 350 lb. For the sake of convenience, doses of 5 c.c., 20 c.c. and 50 c.c. were given, which, for practical purposes, are near enough to the doses defined above in terms of gm./100 Kg.

vaccinated for this. The results, however, were so clear before the eighteenth day that this last test was unnecessary. The results are summarised in Table IV.

The results incorporated in this table are not difficult to assess. The main outcome is that " fresh " vaccine, of both the types tested, produces, even with low doses, a fairly high degree of immunity within three days, and an absolutely solid immunity within eight days, of vaccination. From the mere protocols it would appear that vaccine stored for four months at 0° C. is slightly more efficient than that stored at 37° C. for three days,

TABLE IV.

IMMUNITY INTERVAL FOLLOWING VACCINATION WITH RANGES OF DOSES OF " FRESH " GLYCERINISED SPLEEN.

Vaccine Sample.	*Interval Vaccine to Test.*	*Doses of Spleen per 100 Kg.*	*Bull No.*	*Symptoms.*	*Result.*
Three days at 37° C.	3 days	1 gm.	802	? Fever, few ulcers in mouth 9th to 12th days. ? ? diarrhœa	Survived
			804	Severe cattle plague	Died
		4 gm.	786	No symptoms	Survived
			789	„	„
		10 gm.	755	No symptoms	Survived
			765	Slight fever, 4th to 10th days, few ulcers in mouth, 8th to 10th days	„
		Controls	808	Severe cattle plague	Died
			815	„ „	„
			823	No symptoms (naturally immune)	Survived
	8 days	1 gm.	805	No symptoms	Survived
			809	Fever with acute *Theileria* invasion, but 15th to 18th days only	„
		4 gm.	790	No symptoms	Survived
			791	„	„
		10 gm.	780	No symptoms	Survived
			784	„	„
		Controls	816	Severe cattle plague	Died
			817	No symptoms (naturally immune)	Survived
			818	Severe cattle plague	Died
	13 days	1 gm.	810	No symptoms	Survived
			811	„	„
		4 gm.	798	No symptoms	Survived
			799	„	„
		10 gm.	781	No symptoms	Survived
			782	„	„
		Controls	819	Severe cattle plague	Died
			821	„ „	„

TABLE IV—(*Continued*).

Vaccine Sample.	*Interval Vaccine to Test.*	*Doses of Spleen per 100 Kg.*	*Bull No.*	*Symptoms.*	*Result.*
Four months at 0° C.	3 days	1 gm.	877	No symptoms	Survived
			879	„	„
		4 gm.	867	No symptoms	Survived
			869	„	„
		10 gm.	847	No symptoms	Survived
			858	„	„
		Controls	932	Severe cattle plague	Died
			933	„ „	„
			934	„ „	„
	8 days	1 gm.	978	No symptoms	Survived
			866	„	„
		4 gm.	870	No symptoms	Survived
			873	„	„
		10 gm.	859	No symptoms	Survived
			862	„	„
		Controls	883	Severe cattle plague	Died
			888	„ „	Survived
			893	Acute early symptoms of cattle plague. Beast slaughtered 7th day for further vaccine production	—
	13 days	1 gm.	890	No symptoms	Survived
			901	„	„
		4 gm.	874	No symptoms	Survived
			876	„	„
		10 gm.	864	No symptoms	Survived
			865	„	„
		Controls	935	Severe cattle plague	Died
			936	Acute early cattle plague. Slaughtered for vaccine, 7th day	—
			937	Severe cattle plague	Died

but it would be unwise to apply these small scale results too literally, since the apparent superiority of one sample over the other is at least as probably due to individual variability in the cattle as to difference in the quality of the vaccine.

The next factor for consideration is the dose. Here again there is no evidence that the larger doses produced an appreciably more solid immunity in a given time, or immunity of any degree more rapidly, than the smallest dose tested. This is a most important observation, and, as will be seen in later sections of this paper, it is substantially confirmed in all subsequent experiments. It

indicates that essentially qualitative rather than quantitative factors are at issue. It is of course always possible that a very large scale experiment might show some small balance of evidence in favour of the larger dose, and reference to the much larger experiments in the later section on storage, etc., will show that the quantitative factor is not *entirely* absent. Nevertheless, within the range of normal practice it is permissible to regard the size of the dose (subject, of course, to its being above the effective minimum) as having virtually no effect on the immunity interval.

However, the foregoing experiment was primarily designed with the object of defining some kind of standard for " fresh " vaccine, in order later to attempt to detect and assess deterioration in stored vaccine. It can now be claimed that a sufficiently accurate standard has been determined, at any rate until experiments on stored vaccine show whether it is necessary to be more accurate. The standard is that a dose of 1 gm. spleen per 100 kg. of beast produces within eight days such immunity that the great majority at least of vaccinated beasts develop no symptoms whatsoever when tested with virus. It may be argued that, although the immunity interval is here defined with sufficient accuracy, the dose is not so defined; a smaller dose may in fact produce as sound an immunity within a week. The reply to such objection is that (*a*) the size of the dose appears to have no appreciable effect on the rate of immunity production, and (*b*) unless it is ascertained that with a " stored " vaccine no larger dose is required to produce immunity in a standard time than with a " fresh " vaccine, there is no point in titrating down to the lowest dose. If the necessity is then indicated the titration can then be done.

In pursuance of the above conclusions, every subsequent sample of spleen vaccine, used for whatever purpose in this laboratory, has been checked when fresh to confirm that a dose of 1 gm./100 kg. will produce solid immunity in a week, and in every case it has done so.

Stored Vaccine: Dosage and Immunity Interval.

In field practice, perfectly fresh vaccine, such as was studied in the foregoing section, can rarely be used; after preparation it must almost inevitably be " stored " for a shorter or longer period. The conditions of storage are potentially very variable, but actually call for consideration of two factors only, namely time and temperature.

Having already determined the immunity interval required by " fresh " vaccine, it seemed that deterioration on storage might, if susceptible of quantitative estimation, be demonstrable in two possible ways: (*a*) with a standard dose, the immunity interval might lengthen with progressive deterioration, or (*b*) a progressively larger dose might be required in order to produce immunity in a standard time. The temperature of storage would also be a

potent factor, and would have to be taken into account. Arising out of the foregoing, it might prove possible not only to estimate the nature and rate of deterioration but also to compensate for it in field practice, either by using more than minimal doses or by allowing for a longer immunity interval. The former of these would obviously be preferable.

The line of experimentation was thus to prepare a large mixed sample of vaccine, to store portions of it at different temperatures, and periodically to test samples as to the time required for each sample to produce immunity, using a range of dosages in each test.

This general line was self-evident, but there was difficulty in deciding on the approximate range over which to work. In regard to temperature, it was decided to store at 0° C. and 37° C. only, the former being a routine possibility in the laboratory and the latter fairly representative of shade temperatures in the field throughout most of the year. The real difficulty was in deciding on convenient intervals for the periodical testing of stored samples, and on the doses to use.

A study of the literature was not very profitable. For example, Kakizaki (1918) recorded the efficacy of glycerinised spleen pulp stored at " room temperature " for over 100 days, but multiple, and in some cases very large, doses of vaccine were used, and no comparison was made with fresher samples of the same material. Similar remarks are applicable to his later publications. There is no doubt, however, that large doses of vaccine were effective after relatively long periods of storage, but there is no evidence to show that smaller ones would not have been equally as effective. Later, however, discussing storage alone, Kakizaki *et al.* (1927) claim without going into detail, that the vaccine can be stored in the cold for two-and-a-half to three-and-a-half years, but apparently not for four-and-a-half years. In the case of *formalinised* glycerinised spleen, Evans (1932) found doses of 2 gm. effective for up to 19 weeks' storage at room temperature. Single bulls were, however, used in his experiments and this period should not be too literally accepted.

Similarly, with simple formalinised spleen no precise conclusions can be drawn. Curasson and Delpy (1926) suggest that the keeping quality is poor. They go so far as to suggest that the failure of one or two grammes to protect, whereas 5 gm. succeeded, may have been due to the age of the vaccine (15 days at room temperature). But as all their conclusions, on this as on other factors, are based on the observation of a total of seven calves, they cannot be regarded as final. Later (1929, 1) they reverse this view and suspect that it deteriorates slowly, the reason for the new view being that with one sample of spleen, 5 gm. of the fresh product produced immunity in eleven days whereas after 18 and 47 days' storage this dose failed but 10 gm. succeeded. It is, in

fact, implied that one may compensate for deterioration by giving large doses. Unfortunately, however, after 76 days' storage 5 gm. again succeeded, but this fact is rather lost sight of in their argument. In any case, as in the earlier experiments, only one or two calves were used for each dose, and the possibility of some of them being immune is not considered. Again (1929, 2) they say, based on these same results, that the vaccine "*se conserve assez longtemps.*" Missenard and Zylbertal (1934) say it can be stored for five weeks. It is interesting to note the wide divergence of opinion among Turkish workers who have followed Curasson and Delpy's technique. Tahsin (1933) claims that the vaccine keeps at room temperature for 15 months without deterioration, while Ismail and Zühdi (1933) say it will not keep for ten days.

British workers with formalinised vaccine have published nothing of note on the conservation of vaccine in cold storage, but agree that it does not retain its antigenic value for long periods at room temperature. The longest period claimed is by Hall (1930, 1931), namely, over 72 but under 102 days, but small numbers of cattle were used. Evans (1932), working with formalinised *glycerinised* vaccine, estimates over 19 weeks (133 days) but under 23 weeks (161 days). Conversely, Daubney (1928) doubts whether plain formalinised vaccine keeps for more than a few days.

This rapid survey of the literature, if not complete, is fairly representative. If any conclusions can be drawn therefrom, they are that (*a*) formalinised vaccine cannot safely be stored at " room temperature " (a very variable factor) for more than from several days to a few weeks, (*b*) there are insufficient data on which to express any opinion as to its conservation in cold storage, and (*c*) glycerinised vaccine promises to retain its antigenic value longer than formalinised. In any case, at the time one's own experiments were designed no strong indication existed—and indeed none yet exists—as to the range over which to work. It was therefore necessary to do one's own preliminary ranging.

Preliminary Experiment of 16.12.28.

This was a continuation of the preliminary experiment already described in observations on Safety Interval. A vaccine that had already been incubated at 37° C. for ten days was found to immunise two young bulls at doses of 1 gm. spleen/100 kg., the interval allowed being 20 days. A sample was later sent to Malakal to be tested on young Nuer bulls—the most uniformly and highly susceptible type in the Sudan. At the time of testing, the vaccine had, since becoming safe, been stored on ice for 16 days and at shade temperature of about 37° C. for 60 days. One young bull was given 5 c.c. (1 gm. spleen/100 kg.) and one 25 c.c. (5 gm./100 kg.) and both were tested with controls 30 days later. The controls all (five) developed acute cattle plague, and

were bled out for hyperimmunising virus, while both vaccinated bulls only showed a slight rise of temperature between the fourth and eighth days. A further sample of the same vaccine was similarly tested after one year's storage at room temperature (30° C. to 40° C.) and was found to be useless.

It thus seemed that the period to be studied, in the case of storage at about 37° C. would lie between two months and one year from the time of preparation.

Preliminary Experiment of 5.11.31.

A similar preliminary experiment was carried out on the storage capacity of a sample kept in cold storage. The observations were actually in continuation of those recorded in Table I (Safety Interval) on a sample stored at several degrees above 0° C., more efficient cold storage not being at that time available. The antigenic efficacy of this sample was further tested after periods of eight-and-a-half and 15 months, the details and results being shown in Table V.

TABLE V.

PRELIMINARY TEST OF ANTIGENIC VALUE OF A SAMPLE OF VACCINE (SAMPLE OF 22.2.31) KEPT IN "FAIRLY COLD STORAGE" FOR 8½ AND 15 MONTHS. INTERVAL BETWEEN VACCINATION AND TEST, 26 DAYS IN EACH CASE.

Period of Storage.	*Dose.*	*Bull No.*	*Symptoms.*	*Result.*
8½ months	5 c.c.	722	Nil.	Survived
		723	„	„
	20 c.c.	724	Nil.	Survived
		727	„	„
	50 c.c.	728	Nil.	Survived
		731	„	„
	Controls	754	Severe cattle plague	Died
		764	„ „	„
		776	„ „	„
15 months	5 c.c.	844	Nil.	Survived
		852	„	„
	20 c.c.	856	Nil.	Survived
		857	„	„
	50 c.c.	853	Nil.	Survived
		866	„	„
	Controls	941	Moderate cattle plague	Survived
		942	„ „	„
		943	Severe cattle plague	„

The results generally speaking are sufficiently clear cut. The sample of vaccine that had been kept at a good deal lower temperature than the earlier one retained its vaccinating power for

at least 15 months. Nevertheless, the following criticisms may be offered :—

(*i*) Strict comparison between the vaccines stored at " room temperature " and in " fairly cold storage " is not permissible, since two separate samples were used, and both of these were made from relatively few spleens. Definitive experiments would have to be carried out either with a single bulk stock or with samples made from a relatively large number of spleens.

(*ii*) The period of 15 months over which one sample was stored was the total period of storage after preparation. Vaccine in cold storage does not become safe for use until stored for several weeks. A definitive experiment should be carried out, using a single sample of *safe* fresh vaccine, calculating the period of storage from the time it became safe for use.

(*iii*) The interval at which the vaccinated cattle in Table V were tested for immunity was in both cases 26 days. When the first test (8½ months' storage) was carried out, it was not known how long " fresh " vaccine required to produce immunity, otherwise the interval would have been reduced to a week. For comparative purposes, the interval in the second case (15 months' storage) was also allowed to reach 26 days. In a definitive experiment all immunity tests should be carried out at a week's interval after vaccination if there is to be any chance of studying a possible association between deterioration and lengthening of the immunity interval.

(*iv*) Neither " cold storage " nor " room temperature " was determined other than vaguely. For preliminary experiments of this nature this is not very objectionable, but in a definitive experiment these terms should be stated with precision.

In spite of these critisisms, however, the general indications were good enough ; the antigenic value of fresh safe vaccine stored at, say, 37° C., should be definitively studied over a period of more than two months and less than a year, while vaccine stored at 0° C. should be similarly studied for at least a year.

Definitive Experiment of 12.6.32.

Arising out of the foregoing preliminary observations a definitive experiment was designed as follows :—

1. Prepare a large sample of vaccine from the spleens of several bulls.
2. After mixture, incubate the whole at 37° C. for three days to render safe for use.
3. At this point check original antigenic value by ensuring that 1 gm./100 Kg. produces immunity in a week.
4. Bottle and seal as for routine issue, and divide into two portions, for storage at 37° C. and 0° C. respectively.
5. Periodically titrate samples from each portion by vaccinating cattle at standard (1 gm./100 Kg.) and larger doses, testing their immunity at minimum (1 week) and longer intervals. This form of titration to continue until it is evident whether deterioration is appreciable through (*a*) the standard dose requiring more than the minimum interval to produce immunity, and/or (*b*) a progressively larger dose being required in order to produce immunity in minimum time.
6. Further procedure to depend on the outcome of the above, the intention being that if (*a*) and/or (*b*) materialise the results can be applied

to field practice, whereas if neither does the minimum dose of spleen which will produce immunity in one week must be ascertained.

A sample of vaccine was therefore prepared from twelve spleens, and the final mixture was incubated at 37° C. for three days. Three young bulls were injected at the rate of 1 gm. spleen/100 kg., and their immunity was tested one week later, the result of the test being :—

	Bull No.	*Symptoms.*	*Result.*
Vaccinated ...	875	Nil.	Survived
	880	„	„
	892	„	„
Controls	907	Nil.	Immune
	944	Severe cattle plague	Survived
	945	„ „	Died

At this point arose the difficulty of deciding on what intervals to select for further periodic testing. To test at short intervals would be wasteful, whereas to test at long ones might entail failure to get the desired information in the case of the vaccine stored at 37° C. It was ultimately decided to carry out the first parallel test after six months' storage, the doses being 1 gm., 4 gm. and 10 gm. per 100 kg., and the intervals one and three weeks. The outcome is summarised in Table VI.

The data shown in this table are so self-explanatory that little discussion is demanded. From the purely graphic standpoint one would have wished that the controls to the second half of the test

TABLE VI.

Test of Samples of Glycerinised Spleen Vaccine stored at 0° C. and 37° C for Six Months.

Test Interval.	*Temp. of Storage.*	*Dose. gm./100 Kg.*	*Bull No.*	*Symptoms.*	*Result.*
1 week	0° C.	1	984*	Slight temperature reaction, 1st to 7th days (see footnote)	Survived
			986	No symptoms	„
			987	„	„
		4	994	No symptoms	Survived
			999	„	„
			1000	„	„
		10	1010	No symptoms	Survived
			1011	„	„
			1014	„	„

* Bull 984 was a very wild young beast. It is fairly certain that his fever, occurring during the *first few days* he was being handled, was due to excitement and not to cattle plague.

TABLE VI—(*Continued*).

Test Interval.	*Temp. of Storage.*	*Dose gm./100 Kg.*	*Bull No.*	*Symptoms.*	*Result.*
1 week	37° C.	1	959	Moderate cattle plague	Survived
			960	Severe cattle plague	Died
			961	No symptoms	Survived
		4	966	No symptoms	Survived
			967	„	„
			968	„	„
		10	975	No symptoms	Survived
			976	Severe cattle plague	Died
			977	No symptoms	Survived
		Controls	1020	Severe cattle plague	Died
			1024	„ „	„
			1025	Moderate cattle plague	Survived
3 weeks	0° C.	1	989	No symptoms	Survived
			990	„	„
			991	Thermal reaction only	„
		4	1001	No symptoms	Survived
			1003	„	„
			1004	„	„
		10	1015	No symptoms	Survived
			1018	Intercurrent disease after vaccination, immunity not tested	—
			1019	No symptoms	Survived
	37° C.	1	962	No symptoms	Survived
			963	Severe cattle plague	Died
			965	„ „	Survived
		4	970	No symptoms	Survived
			971	Severe cattle plague	„
			973	Moderate cattle plague	„
		10	978	No symptoms	Survived
			982	Moderate cattle plague	„
			983	No symptoms	„
		Controls	1032	No symptoms	Survived
			1033	„	„
			1034	Moderate cattle plague	„

had been more highly susceptible. The behaviour of some of the vaccinated cattle, however, proved what is normally expected of controls, *viz.*, the virulence of the test virus.

In the first place, the results confirm what was roughly shown in the preliminary tests, namely that vaccine stored at 37° C. deteriorates more rapidly than that stored at 0° C. The cold storage sample in this experiment proved in no perceptible way inferior to the freshly prepared product, but the sample stored at 37° C. had already deteriorated beyond a point at which it could

be issued for field use. Furthermore, it cannot be said that larger doses or a longer interval were superior to the smallest dose and the minimum interval. In the cold storage sample no deterioration of any kind was perceptible, while the incubated sample seems to have already deteriorated beyond a point at which larger doses or a longer interval could show their superiority, even if this had at some period been demonstrable.

One unfortunate feature of this experiment was that the time of this first test was badly chosen. Vaccine stored at 37° C. obviously had to be studied at an earlier stage and another sample could not be studied in exact parallel with one stored at 0° C. unless an entirely new experiment were instituted. This did not seem justifiable on account of expense. It was therefore decided to continue the periodic tests of the sample in cold store, and to make a fresh bulk stock from several cattle for study in storage at 37° C. By making this from the spleens of several cattle, the possible criticism that the two vaccines might have different properties would be for practical purposes eliminated.

The further testing of the cold storage stock was continued at half-yearly intervals for two-and-a-half years. Sufficient stock was originally made to continue the experiment for five or six years if necessary, but, as will shortly be seen, there seemed to be no point in continuing so long. The results of the further periodic testings of this cold-storage sample are shown in Table VII.

TABLE VII.

FURTHER TITRATIONS OF GLYCERINISED SPLEEN VACCINE STORED AT 0° C.

Period of Storage.	*Interval.*	*Dose g/Kg.*	*Bull No.*	*Symptoms.*	*Result.*
1 year	1 week	1	1107	No symptoms	Survived
			1111	? fever, 3rd to 5th days	„
			1114	No symptoms	„
		4	1094	No symptoms	Survived
			1095	„	„
			1099	„	„
		10	995	No symptoms	Survived
			1002	„	„
			1081	„	„
		Controls	1195	Severe cattle plague	Died
			1196	„ „	„
			1197	„ „	„
			1198	„ „	„
	3 weeks	1	1115	No symptoms	Survived
			1116	„	„
			1121	Slight fever, 6th to 10th days only	„
		4	1102	No symptoms	Survived
			1104	„	„
			1106	„	„

TABLE VII—(*Continued*).

Period of Storage.	*Interval.*	*Dose g/Kg.*	*Bull No.*	*Symptoms.*	*Results.*
1 year	3 weeks	10	1083	No symptoms	Survived
			1087	,,	,,
			1093	,,	,,
		Controls	1257	Acute early thermal reaction	(Slaughtered
			1258	,, ,,	for
			1259	,, ,,	vaccine)
1½ years	1 week	1	1418	Severe cattle plague	Died
			1419	No symptoms	Survived
			1427	Fever, 4th to 7th days only	,,
		4	1421	No symptoms	Survived
			1428	,,	,,
			1429	,,	,,
		10	1420	No symptoms	Survived
			1422	Slight fever, 4th to 6th days only	,,
			1423*	Slight fever, 3rd to 6th days. Half-a-dozen pin-head buccal vesicles 9th day, healed 10th day (see footnote)	,,
		Controls	1450	Severe cattle plague	Died
			1466	No symptoms	Immune
			1467	Severe cattle plague	Died
	3 weeks	1	1433	Slight fever, 11th to 15th days, few buccal ulcers, 11th to 13th days	Survived
			1434	Fever, catarrh and few buccal ulcers, all 9th to 12th days	,,
			1435	No symptoms	,,
		4	1430	Fever, 5th to 15th days	Survived
			1431	No symptoms	,,
			1432	,,	,,
		10	1424	Fever, 1st to 5th days with *Theileria* in blood	Survived
			1425	No symptoms	,,
			1426	,,	,,
		Controls	1498	No symptoms	Immune
			1499	Severe cattle plague	Died
			1500	,, ,,	,,
2 years	1 week	1	1639	No symptoms	Survived
			1642	Fever, 4th to 13th days. Few buccal ulcers, 11th to 12th days	,,
			1677	No symptoms	,,
			1680	Fever, 6th to 12th days. Appreciable buccal ulceration, 11th to 13th days; mild diarrhœa, 11th to 14th days. Off feed part of 11th day only	,,

* Bull 1423 developed an abscess at the site of injection. Possibly suppuration delayed absorption.

TABLE VII—(*Continued*).

Period of Storage.	*Interval.*	*Dose g/Kg.*	*Bull No.*	*Symptoms.*	*Result.*
2 years	1 week	4	1618	No symptoms	Survived
			1649	,,	,,
			1671	,,	,,
			1675	,,	,,
		10	1656	No symptoms	Survived
			1672	Highest temperature, 101·8°, 6th morning	,,
			1678	Slight fever, 5th to 8th days	,,
			1683	No symptoms	,,
		Controls	1693	Severe cattle plague	Died
			1694	,, ,,	,,
			1695	,, ,,	,,
			1696	,, ,,	,,
	3 weeks	1	1615	No symptoms	Survived
			1637	Morning temperature, 102° on 8th and 9th days	,,
			1645	No symptoms	,,
			1647	Marked fever, 5th to 9th days, few scattered buccal ulcers, 8th day; healed 11th day	,,
		4	1638	No symptoms	Survived
			1650	,,	,,
			1676	,,	,,
			1679	Marked fever, 6th to 9th days only	,,
		10	1655	No symptoms	Survived
			1657	,,	,,
			1658	,,	,,
			1681	,,	,,
		Controls	1701	Severe cattle plague	Died
			1702	,, ,,	,,
			1703	,, ,,	,,
			1704	No symptoms	Immune
2½ years	1 week	1	1726	Marked fever, 4th to 9th days, catarrh, 8th day, several discrete buccal ulcers, 6th to 10th days	Survived
			1727	? ? thermal reaction	,,
			1751	Fever, 4th to 8th days only	,,
			1752	,, 5th to 8th days. Half-a-dozen pin-head ulcers only	,,
		4	1716	No symptoms	Survived
			1717	,,	,,
			1719	,,	,,
			1723	,,	,,
		10	1714	No symptoms	Survived
			1718	,,	,,
			1728	,,	,,
			1738	,,	,,
		Controls	1712	Severe cattle plague	Died
			1713	,, ,,	,,
			1732	No symptoms	Immune
			1757	Severe cattle plague	Died

TABLE VII—(*Continued*).

Period of Storage.	*Interval.*	*Dose g/Kg.*	*Bull No.*	*Symptoms.*	*Result.*
2½ years	3 weeks	1	1729	Fever, 4th to 7th days only	Survived
			1750	„ 5th to 9th days, few buccal ulcers, 9th and 10th days	„
			1754	No symptoms	„
			1755	„	„
		4	1724	No symptoms	Survived
			1728	„	„
			1730	„	„
			1731	„	„
		10	1720	No symptoms	Survived
			1722	„	„
			1746	„	„
			1756	„	„
		Controls	1734	Severe cattle plague	Died
			1735	No symptoms	Immune
			1736	Severe cattle plague	Died
			1740	„ „	„

The first, and most striking, general conclusion that can be drawn from this table is that even after two-and-a-half years' storage the vaccine was still of high antigenic value, since even at the lowest dose it produced in nearly every case a very serviceable immunity within the minimum period. Throughout the whole series of tests only one vaccinated beast died (No. 1418). No other vaccinated beast ever aroused any fear that it might die, or even be seriously ill; in fact, without the regular use of a thermometer and the most careful clinical examination, only two other bulls, Nos. 1680 and 1726 (one of which was slightly off feed with loose fæces, while the other developed slight catarrh), would have shown any signs of sickness whatsoever.

To pass on to the more particular information that was being sought, namely, whether deterioration on storage could be detected or assessed by observing that a larger dose might be effective after the smallest one failed to immunise, or whether, when immunity would not be produced in the minimum interval, it would develop after a longer one. It is immediately evident that the first of these questions can be answered in the affirmative. Vaccine stored for one year may be dismissed as being, within the range of the titration, almost, if not quite, as efficacious as when first prepared. It is perhaps not wise to attach too much importance to isolated febrile reactions, which might not even be specific, but it must nevertheless be observed that the only two cattle which developed even such slight fever were those which received the smallest dose of vaccine. The one beast which died, and the only other two

which showed any visible clinical symptoms (Nos. 1418, 1680 and 1726), were also among those which received the smallest dose. Furthermore, in all samples of vaccine stored for one-and-a-half years or more the cattle showing the most definite symptoms, mild though these may have been, were those which had received the 1 gm./100 kg. dose. Symptoms developed by cattle receiving any but the lowest dose were so rare and so slight that they afford no further information on this point.

Regarding the second question, *viz.*, whether in a partially degraded vaccine a sounder immunity will develop after a longer interval, the answer can in no way be so easily given in the affirmative. In principle, allowing for individual variation in the cattle, the data are strictly not sufficient to permit any conclusion at all. Nevertheless, many cattle were used, and it cannot be denied on close examination of the protocols that some small balance of evidence is in favour of a slightly longer interval allowing a rather sounder immunity to develop. The evidence at any rate is not to the contrary effect. It may seem that to argue this point is mere hair-splitting, since the phenomenon, even if admitted, is of such low degree as to be of no practical importance. It is, however, considered desirable to at least discuss the records, pending consideration of studies on vaccine samples stored in the incubator. From the more rapid degradation of these, more definite conclusions may possibly be obtained.

Before proceeding to consideration of the latter, observations on the vaccine stored at 0° C. may be summarised as follows :—

(*i*) The rate of deterioration is extremely slow. For a year at least the vaccine in doses of 1 gm./100 Kg. remains for practical purposes as efficacious as absolutely fresh vaccine, and it remains a very useful product for a much longer period.

(*ii*) One phenomenon of deterioration is that, in order to produce immunity in minimum time, larger doses are required. Owing, however, to the extremely slow rate of deterioration this observation cannot at this point be translated into absolute terms.

(*iii*) The balance of evidence in favour of a slightly degraded vaccine producing equally sound immunity after a longer interval (following vaccination) than a fresh sample is extremely slender. Before finally drawing any conclusion, it will be necessary to study more rapidly degraded vaccine, since to continue the study of the cold storage sample might entail a delay of several years.

One may now revert to vaccine stored in an incubator at 37° C. A fresh mixed stock was made from the spleens of six young bulls. This was incubated at 37° C. for three days and tested for original potency by injecting three young cattle at the rate of 1 gm./100 kg. and testing their immunity seven days later. The results of this test were :—

	Bull No.	Symptoms.	Result.
Vaccinated	1441	No symptoms	Survived
	1453	,,	,,
	1456	,,	,,
Controls	1498	No symptoms	Immune
	1499	Severe cattle plague	Died
	1500	,, ,,	,,

A small sample was removed and stored at 0° C. for subsequent check test, and the main stock was stored in an incubator at 37° C. The programme was to carry out monthly titrations of this stock on the same lines as those in Table VII for so long as might prove necessary. It had already been shown that this series of tests would not have to be continued for so long as six months; ultimately they were discontinued after three months. The results are summarised in Table VIII.

TABLE VIII.

Monthly Tests of Glycerinised Spleen Vaccine stored at 37° C., and Check Test of Sample stored at 0° C.

Period of Storage.	Interval.	Dose gm./Kg.	Bull No.	Symptoms.	Result.
1 month	1 week	1	1449	Morning temperature 102° C. 6th and 7th days	Survived
			1451	Slight fever, 4th to 10th days	,,
			1452	No symptoms	,,
		4	1454	Slight fever, 4th to 7th days	Survived
			1455	No symptoms	,,
			1462	,,	,,
		10	1472	No symptoms	Survived
			1473	,,	,,
			1475	,,	,,
		Controls	1514	Severe cattle plague	Survived
			1515	No symptoms	Immune
			1517	Severe cattle plague	Survived
	3 weeks	1	1461	No symptoms	Survived
			1481	,,	,,
			1486	,,	,,
		4	1460	Intercurrent disease, not tested	—
			1471	No symptoms	Survived
			1474	Broke his neck while being secured	—
		10	1476	No symptoms	Survived
			1474	,,	,,
			1484	,,	,,
		Controls	1522	Severe cattle plague	Died
			1523	No symptoms	Immune
			1530	Severe cattle plague	Died

TABLE VIII—(*Continued*).

Period of Storage.	*Interval.*	*Dose gm./Kg.*	*Bull No.*	*Symptoms.*	*Result.*
2 months	1 week	1	1442	No appreciable fever, but two pin-head buccal ulcers 7th day; healed 10th day	Survived
			1477	? ? Thermal reaction	,,
			1480	Fever, 8th to 10th days only	,,
			1482	No symptoms	,,
		4	1487	Morning temperature, 102° C., 4th and 5th days	Survived
			1488	Fever, 4th to 6th days	,,
			1489	,, 4th to 7th days. Three pin-head buccal ulcers 7th day; healed 10th day	,,
			1490	Morning temperature, 102° C., 4th and 5th days	,,
		10	1478	No symptoms	Survived
			1513	,,	,,
			1519	,,	,,
			1525	,,	,,
		Controls	1539	Severe cattle plague	Died
			1540	,, ,,	,,
			1541	,, ,,	,,
			1542	,, ,,	Survived
	3 weeks	1	1483	Slight fever, 4th to 9th days only	Survived
			1485	Severe cattle plague	Died
			1520	No symptoms	Survived
			1532	,,	,,
		4	1492	No symptoms	Survived
			1493	,,	,,
			1518	,,	,,
			1521	Severe cattle plague	Died
		10	1526	No symptoms	Survived
			1528	,,	,,
			1529	,,	,,
		Controls	1572	Severe cattle plague	Died
			1573	,, ,,	,,
			1574	,, ,,	,,
			1575	,, ,,	,,
3 months	1 week	1	1538	Fever, 4th to 8th days only	Survived
			1544	No symptoms	,,
			1545	Fever, 3rd to 10th days. Considerable buccal ulceration. No diarrhœa	,,
			1546	Fever, 4th to 8th days only	,,
		4	1536	Fever, 4th to 8th days only	Survived
			1537	No symptoms	,,
			1543	Severe cattle plague	Died
			1549	Fever, 5th to 8th days only	Survived
		10	1491	Fever, 4th to 6th days only	Survived
			1533	,, 3rd to 8th days only	,,
			1534	No Symptoms	,,
			1535	Fever, 5th to 7th days only	,,

TABLE VIII—(*Continued*).

Period of Storage.	*Interval.*	*Dose gm./Kg.*	*Bull No.*	*Symptoms.*	*Result.*
3 months	1 week	Controls	1580	Thermal reaction only	Survived
			1581	Severe cattle plague	,,
			1583	,, ,,	Died
	3 weeks	1	1552	Fever, 5th to 11th day, mild stomatitis, mild diarrhœa. Never off feed and no loss of condition	Survived
			1554	No symptoms	Survived
			1559	Fever, 4th to 8th days only	,,
			1564	Severe cattle plague	Died
		4	1553	Fever, 5th to 9th days only	Survived
			1560	No symptoms	,,
			1562	Fever, 4th to 7th days only	,,
			1563	,, 5th to 10th days only	,,
		10	1547	No symptoms	Survived
			1550	Fever, 4th to 8th days only	,,
			1558	No symptoms	,,
			1561	,,	,,
		Controls	1607	Severe cattle plague	Died
			1608	,, ,,	Survived
			1609	,, ,,	Died
			1610	,, ,,	,,
Check sample 5 months at 0° C.	1 week	1	1566	Slight fever, 4th to 8th days only	Survived
			1571	,, 4th to 6th days only	,,
			1644	? ? thermal reaction	,,
			1646	No symptoms	,,
			1648	,,	,,
			1651	,,	,,
		Controls	1665	Severe cattle plague	Died
			1666	,, ,,	,,
			1667	,, ,,	,,

A general perusal of this table shows that, while the check sample stored at 0° C. retained its original value almost unimpaired, the vaccine stored at blood-heat arrived in two or three months at much the same stage of degradation as the freezing point sample in the preceding experiment did in two-and-a-half years. After one month's storage it was nearly, but possibly not quite, as efficacious as when freshly prepared. After two and three months' storage the vaccine still retained much of its value, but permitted occasional casualties in the cattle receiving the 1 gm. and 4 gm./100 kg. doses.

The slightly greater value of a larger dose is again seen, particularly in the tests after one and two months. Many of the 1 gm./100 kg. beasts, and some of the 4 gm./100 kg., developed symptoms, but none receiving 10 gm./100 kg. did so. The phenomenon is not so evident in the three months' test.

In these titrations, slightly more than in the earlier ones of the cold-storage samples, the greater value of a longer interval is also mildly evident, although individual variation in the cattle—apparently a much more important factor—largely masks it. Taken together with the evidence obtained in the case of the cold storage sample, it is certain that a partially degraded vaccine does produce a slightly stronger immunity after a longer interval, but, as was fairly evident from the first series of experiments, this feature is of negligible importance.

It is thus seen that the nature of the effects of storage are the same, whether the vaccine be stored at blood heat or at freezing point, although the changes occur more rapidly with higher temperature. It is therefore desirable to draw one's conclusions and discuss their significance from the data of both series of experiments jointly. These are as follows :—

1. Glycerinised spleen vaccine deteriorates much more rapidly at high than at low temperatures. In order to decide on how long it may be stored, either in cold storage (0° C.) or at Sudan shade temperatures (up to 37° C.), one must have a clear idea of what the vaccine is supposed to accomplish. If it must produce so high a degree of immunity at a dose of 1 gm./100 Kg. that no vaccinated cattle must succumb or develop appreciable symptoms on subsequent exposure to cattle plague (neglecting for the present the duration of immunity), it may not be safely stored for much more than a month under ordinary field conditions, or a year in cold storage. If, however, the vaccine is destined merely to prevent the occurrence of epizootics, and a few odd casualties will be of little account, then it can be stored for double these periods. The former objective is, of course, preferable, and the vaccine can withstand storage sufficiently well for it to be as a rule attainable. Nevertheless, especially in the Sudan with its small veterinary staff, its long distances, indifferent transport, and nomadic cattle-owning tribes, a *pis aller* is often all that is possible.

2. The immunity produced is evidently more referable to qualitative than to quantitative factors in the vaccine. Thus, when a sample has deteriorated to the degree that a small dose will not produce a sound immunity in minimum time, a relatively great (for routine purposes impracticably great) increase in dosage is necessary to compensate for it. Even so, owing to the great individual variation in cattle, it is not certain that this compensation will be universally demonstrable.

3. A similar conclusion can be drawn in regard to the immunity interval. If, with a slightly degraded vaccine, a given degree of immunity does not develop in one week, as occurs with fresh vaccine, the evidence that it will do so after a slightly longer interval is so slender as to be negligible.

4. Arising out of the foregoing conclusions, it is evident that the policy should be to adopt approximately the minimum dose as the one for routine use—allowing perhaps a small excess as a matter of general usage. It should be recognised that this dose will produce immunity in a week, and that when the vaccine has been stored for, say, two months under " field " conditions or two years in cold storage, it will fail to do so in a progressively increasing number of cases. If the vaccine is being used under the more usual Sudan conditions, where the prevention of large outbreaks is the primary consideration, some small latitude may be permitted as to the period of storage, but

if it is being used to vaccinate cattle which are destined to move along trade routes, and particularly cattle destined for export, no latitude whatsoever should be allowed. It should, further, be recognised that in a vaccine that has been stored for more than the permissible period, there will be no practical justification for attempting to compensate for deterioration by giving slightly larger doses, nor should it be expected that immunity will develop after a slightly longer interval than the minimum.

Before implementing this policy it will be necessary to determine the minimum dose of fresh vaccine that will produce immunity in one week. This determination will be the subject of a later section of this paper, when other tissues besides spleen will be studied.

Duration of Immunity

On this subject there is, on the whole, much less divergence of opinion expressed in earlier publications than on any of the other main factors at issue.

To deal first with glycerinised spleen, Kakizaki (1925) gives two tables (XI and XII) summarising experiments to determine this point. In the first series he claims that immunity persists for at least a year, but one observes that infection tests carried out at any interval of five months or more were second infection tests, since all vaccinated cattle had been submitted to a preliminary infection test ten or 15 days after vaccination. The results of this series of tests are thus invalidated. In the second series original infection tests of vaccinated cattle were carried out at intervals of five and six months (two beasts in each case), and all survived. Two doses of vaccine had also been given, but this probably did not affect the issue. Kakizaki, Nakanishi and Oizumi (1927) publish some field observations which would have been more informative if recorded in greater detail. It seems, however, that large numbers of vaccinated cattle exposed to cattle plague between five and nine months after vaccination all survived with the exception of one heavily lactating cow (nine months' interval). Kakizaki, Nakanishi and Nakamura (1928) give further (? including also some of the preceding) data of natural exposure of vaccinated cattle at intervals of up to seven months, recording death-rates of only one or two per thousand. Unfortunately, more precise laboratory observations on simple glycerinised vaccine are not available.

In the case of *formalinised* glycerine, Evans (1932), using fairly large doses, tested small numbers of cattle periodically up to a period of 49 weeks after vaccination and observed no serious symptoms. Slight fever, however, was a fairly usual sequel to the tests.

A good deal of information is available about simple formalinised vaccine, the following being typical examples: Daubney (1930) claims 15 months. This statement seems to be a general

one as the result of field experience, as it is not supported by detailed protocols. Curasson (1931) says that, in the field, cattle exposed to infection after any interval of more than six months may contract the disease. He quotes three specific instances where (i) ten out of 320 at seven months' interval, (ii) 14 out of 125 at nine months, and (iii) nine out of 81 at nine-and-a-half months did so. The death-rate is not given, but he says that all do not *necessarily die,* thus implying that some do. In another paper Curasson (1934, 2) describes herds vaccinated eight months earlier as being "*entièrement sensibles.*" Hall (1931), using small numbers of cattle in periodical tests, gives the duration as one year. Bergeon and Cèbe (1932), as the result of laboratory tests, conclude that immunity begins to disappear after four months, and recommends revaccination after every six months. Ismail and Zuhdi (1933) estimate seven months, giving no details. Missenard and Zylbertal (1934) record that in natural outbreaks even five months after vaccination cattle may not only contract cattle plague, but may die. Cèbe and Perrin (1934) record a natural outbreak at an interval of four months; the infection rate was two out of 172 with no deaths.

In this short review of the literature no account has been taken of the differences in technique of preparation, dosage, etc., which varied almost from observation to observation, and these may possibly have been to some extent responsible for the variation in conclusions of different workers. In spite of this possibility, however, the findings as a whole are not extremely divergent. One gathers that in the case of simple formalinised vaccine the immunity *begins* to wear off in four or five months, after which interval certain individuals become again susceptible. With increasing interval a larger number become again susceptible, but the end of this process has not been determined (there seems to be no great point in determining it). In the case of glycerinised vaccine less workers can be quoted, but a good deal of material seems to have been studied. A similar falling off in immunity occurs, but according to Japanese observers it seems to start slightly later and possibly progresses more slowly. In any case, the variable susceptibility of the very widely divergent types of cattle used by different workers may have had some influence on the results, and it was decided to study the duration of immunity in Sudan cattle. Only one experiment was carried out, and it was discontinued after ten months for reasons which will presently be shown.

Experiment of 29.6.33.

A mixed stock of vaccine made from five spleens incubated at 37° C. for three days, and checked for original potency by vaccinating two young bulls with 1 gm./100 kg. and testing them with virus seven days later. Neither vaccinated bull developed any symptoms, while two controls developed cattle plague and died.

Twenty-eight young cattle were then given vaccine at the rate of 1 gm./100 kg., and 30 at the rate of 5 gm./100 kg., the larger dose being included with the object of ascertaining whether the size of the dose had any effect on the duration of immunity. The further intention was to test a few of each group as to immunity every few months. As will shortly be seen, tests after four and eight months were carried out, at which no vaccinated beast developed more than entirely negligible symptoms. A few cattle also died of injury or other causes not connected with cattle plague. Ten months after the institution of the experiment one beast showed symptoms of cattle plague, the disease having by some mysterious means gained access from an outbreak that had just been reported some little distance away (the only outbreak within 100 miles since the commencement of the experiment). Since all the cattle were running together in a large enclosure, it was necessary to conclude the experiment by giving them all a dose of virus; to have refrained from so doing would have been to invalidate all subsequent observations, because vaccination *plus* infection would have been at issue. The results of the first two tests are given in Table IX, while those of the final test, which for the sake of economy in space are more conveniently set out in a different style, are given in Table X.

TABLE IX.

IMMUNITY TESTS ON VACCINATED CATTLE FOUR AND EIGHT MONTHS AFTER VACCINATION.

Interval.	*Dose gm./100 Kg.*	*Bull No.*	*Symptoms.*	*Result.*
4 months	1	1059	No fever. doubtful diffuse stomatitis without ulceration, 7th to 10th days	Survived
		1232	No symptoms	„
		1234	„	„
	5	1043	No symptoms	Survived
		1233	„	„
		1242	„	„
	Controls	1416	No symptoms	Immune
		1417	Severe cattle plague	Survived
8 months	1	1283	No symptoms	Survived
		1240	Fever, 4th to 8th days only	„
		1273	No symptoms	„
		1271	„	„
	5	1277	No symptoms	Survived
		1272	Fever, 4th to 8th days only	„
		1276	„ 4th to 9th days only	„
		1307	„ 4th to 8th days only	„
	Controls	1580	Thermal reaction only	Survived
		1581	Severe cattle plague	Survived
		1583	„ „	Died

TABLE X.

IMMUNITY TEST TEN MONTHS AFTER VACCINATION.

Dose gm./100 Kg.	*Total Cattle Tested.*	*Results.*				
		No Symptoms.	*Fever only Survived.*	*Mild Stomatitis Survived.*	*Severe Symptoms Survived.*	*Died.*
1	16*	7	5	3	—	1
5	22	18	2	2	—	—
Controls	3	—	—	—	—	3

* Includes the originally detected naturally-infected case.

When infection was first found to have spread to one's experimental herd, which was certainly in a normally effective state of isolation, a feeling of great annoyance was experienced. Later on, however, as the results of deliberately infecting the whole herd developed, one came to doubt whether it was not perhaps a fortunate occurrence. In its absence, one might have continued on several more occasions to select and test a few cattle before encountering any individual which would develop more than the mildest symptoms, whereas, by being forced when one was to test the whole herd, a truer perspective view was obtained.

The first feature to call for comment among the data in Tables IX and X is the great individual variability of the cattle. This was to be expected, as the same phenomenon had constantly been evident in antecedent titrations of stored vaccines. Consequently, the duration of immunity, no less than the permissible storage period, cannot be discussed in terms which do not include this factor.

Immunity falls away in certain individuals more rapidly than in others, just as a partially degraded vaccine first shows its lower value by failing to immunise certain individuals. From these two tables (neglecting for the present the question of dosage), it is evident that *nearly* all cattle have a strong immunity for at least ten months after vaccination; of 38 cattle tested after this interval, only one developed more than the mildest symptoms, and most of them did not develop even so much as mild fever. Nevertheless, immunity is at this stage beginning to deteriorate, deterioration will be progressive, and an increasing number of cattle will develop symptoms or even die on exposure to infection.

The practical implications in this case again, as was argued in connection with the storage of vaccine, will depend on what one decides that the vaccine must accomplish. If every individual in a herd of vaccinated cattle must remain so solidly immune that no deaths must occur and no serious symptoms develop on subsequent exposure, then the duration of immunity is something short, but probably not much short, of ten months. If, however, the object

is to prevent large epizoötics, and the death of an odd beast or two does not matter, then immunity in general will certainly persist for a good deal more than ten months, and nothing but very large scale field experiments, carried out under very close observation and submitted to statistical treatment, will provide any better answer to the question. At any rate, for general field use in the Sudan it will be fairly conservative to regard vaccinated herds as " immune " for a year after vaccination.

A few words may now be said about the effect of dosage on duration of immunity. It is not necessary to discuss in great detail the data provided in Tables IX and X. It is easily appreciated on quite brief perusal of them that dosage has about as much bearing in this case as it has in compensating for slight deterioration. That is to say, a larger dose does seem to produce a somewhat more durable immunity, but even an impracticably larger dose would not produce a markedly more durable one. It seems therefore that, as in the case of stored vaccines, little practical benefit would follow by using doses much larger than the minimum; in fact, quality and not quantity of vaccine is the critical factor.

Dosage Titration of Spleen and Other Tissues.

From what has gone in the preceding sections, it follows that determination of the minimum dose of spleen that will produce immunity is indicated; other tissues, such as have been studied by other workers, can be simultaneously titrated. The literature on this subject, at any rate in so far as spleen is concerned, has been partially reviewed in the section dealing with dosage, with the general conclusion that it was not very helpful. In the case of glycerinised spleen alone, it may be recalled that Kakizaki and his collaborators proved with a very small number of bulls that 1·5 gm./100 kg. was effective. Smaller doses were not fully tried out, but half this dose protected in a few instances. One's own experiments have already shown, and on quite a large scale, that 1 gm./100 kg. is quite sufficient and that the possibility of using smaller doses should be studied. In the case of formalinised spleen, owing largely to the inadequacy of many of the experiments to provide definitive evidence, estimates (they are often no more) of the minimum dose are more variable. Nevertheless, from the findings which one judges to be the more reliable, it seems that the minimum is not less than 1 gm./100 kg.

A fair amount of literature has recently accumulated on the efficacy of tissues other than spleen. Lymphoid tissues as a class, *e.g.*, lymphatic glands, thymus, tonsils, are reported on with general approval; other tissues such as lung and testicle receive mixed approval; the remaining body tissues are generally regarded as useless. Subcutaneous injection alone is here considered. Curasson, Dischamps and Andryesky (1929), and Curasson and

Zylbertal (1934), claim that the dose can be reduced by injecting intradermally. Their protocols, however, fail to show that the lowest dose by any route has been ascertained.

Fairly comprehensive titrations of glycerinised tissues have been carried out by Kakizaki, Nakanishi and Oizumi (1927) and Kakizaki, Nakanishi and Nakamura (1928). Their conclusions appear to be that the minimum prophylactic doses, translated into terms of grammes of fresh tissue per 100 kg., were : tonsil 0·44, spleen 0·66, thymus 0·88, and lymphatic glands 1·33. They admit that they did not use enough animals to be absolutely certain of these doses.

It would, for example, appear that lung tissue is effective in doses of 0·88 gm./100 kg. and they definitely state that it ranks next in order after spleen, but one must admit that owing to the extreme dissimilarity of spleen (and the other lymphoidal tissues) and lung, this is a dangerous conclusion to draw from the observation of very few cattle.* Nevertheless, these titrations of tissues, which are the first recorded, show great promise even if the protocols are not fully convincing. It has by now become very obvious that no observation on cattle plague vaccination is very convincing unless made on a considerable number of cattle.

It is further relevant to point out that the standard of immunity required by the Japanese workers was that only mild symptoms should develop when vaccinated beasts were subsequently tested with virus. In one's own experiments hitherto one has regarded symptomless immunity as the standard. With the knowledge that the size of the dose is not *entirely* without influence on immunity production, this standard may have to be temporarily suspended while titrating down to the very lowest dose, and certainly so if one's results are to be compared with those of the Japanese workers.

Regarding other types of tissue vaccine, notably the formalinised ones, Curasson and Delpy (1929, 2) use lung mixed with spleen and lymphatic glands, but they use very large doses and do not appear to have titrated the lung. Several other workers, in particular the French school, use lung in their vaccines, but as a rule with no more justification. If lymphoid tissue is the one at issue, as it seems to be, it is frankly difficult to see why lung should be thought to contain more than any other essentially non-lymphoid tissue. However, Jacotot and Colson (1934, 1) record some titrations of lymphatic glands, spleen and lung, using toluolised glycerine as their vehicle, and attempting to find the lowest dosage at which those tissues will prevent (*a*) fever and *catarrhe* and (*b*) diarrhœa. *Catarrhe* is not defined and buccal ulceration is not mentioned, but in spite of this the principle is not difficult to understand. Unfortunately very few cattle are used in their tests and even these are not used in the form of groups of appreciable size. The results are therefore not easy to assess, but

the authors conclude that the dose of glands, spleen and lung should be as 1 : 2 : 4, and, so far as one can gather, no less than 2, 5 and 10 gm. of actual tissue respectively is necessary to prevent *catarrhe* while rather less than half these quantities will prevent diarrhœa. Van Saceghem (1933) claims that testicle is effective at a dosage of 4 gm./100 kg., while Jacotot and Colson (1934, 2) find it useless. It is surprising to find that the foregoing are the most authoritative records of tissue vaccine titration, and one cannot conclude more than that 1 gm./100 kg. of spleen, rather less of lymphatic glands, etc., and possibly very much more of lung, is effective in vehicles other than glycerine.

Fortunately, working as one was with glycerine, the Japanese work afforded a good indication of the range over which to work, and, with the exception of lung, of the tissues to test. The range had obviously to be below 1 gm./100 kg., except possibly in the case of lung. Also, it seemed that, with this possible exception, the only tissues worth studying were the lymphoid tissues such as lymphatic glands, spleen, thymus and tonsil. However, owing to the small weight of the tonsils relatively to that of the lymphatic glands which they so closely resemble, it was decided not to test them separately but to include them with the glands.

Experiment of 9.1.35.

Six young bulls were utilised in making four vaccine samples, which were of (i) spleen, (ii) thymus, (iii) lymphatic glands *plus* tonsils (excluding the mesenteric glands), and (iv) lung. All samples were incubated at 37° C. for three days and injected into groups of cattle in a range of doses.* Seven days after vaccination all vaccinated cattle, together with some controls, were given virus. The results of this test are shown in Table XI.

Two general conclusions can easily be drawn from these results, first that lung tissue is, as was suspected, markedly inferior to the others, and second that the titration of tissues other than lung has not been extended to the end-point. Vaccine made from lung tissue would not protect even against death in doses of 2 gm./100 kg., while all other tissues protected against any but the mildest symptoms in doses of 0·5 gm./100 kg., and almost universally against even mild fever at a dose of 1 gm./100 kg. It will be noticed that one bull that received spleen vaccine at the rate of 1 gm./100 kg. developed fever on being tested. Possibly this was due to the vaccine being somewhat under the usual standard, or it may conceivably have been due to having encountered a particularly susceptible beast; the latter is the more probable explanation. The main issue is in any case not affected. Whether the vaccine was fully, or only barely, up to standard, thymus and lymphatic gland tissue were at least as antigenically

* It was anticipated that the virus in tissues other than spleen would, in identical circumstances, become avirulent in the same time. Such proved to be the case.

potent as spleen, whereas lung tissue equally certainly was not. Unfortunately, however, the experiment did not lead to the determination of the minimum effective dose of the more useful tissues.

TABLE XI.

PRELIMINARY TITRATION OF VARIOUS TISSUES UTILISED IN MAKING GLYCERINISED CATTLE PLAGUE VACCINE. VACCINATED CATTLE TESTED ONE WEEK AFTER VACCINATION.

Tissue.	*Dose gm./100 Kg.*	*Bull No.*	*Symptoms.*	*Result.*
Spleen	0·5	1741	Fever, 4th to 9th days only	Survived
		1742	No symptoms	,,
		1763	Fever, 4th to 9th days only	,,
	1·0	1762	? ? Thermal reaction	Survived
		1772	Fever, 5th to 11th days	,,
		1774	No symptoms	,,
	1·5	1761	No symptoms	Survived
		1778	,,	,,
		1789	,,	,,
Thymus	0·5	1743	Fever, 7th to 9th days only	Survived
		1747	Marked fever, 4th to 11th days. A few buccal ulcers 11th day, healed 14th day	,,
		1766	Fever, 5th to 8th days only	,,
	1·0	1770	No symptoms	Survived
		1775	,,	,,
		1784	,,	,,
	1·5	1782	No symptoms	Survived
		1788	,,	,,
		1803	,,	,,
Lung	0·5	1745	Severe cattle plague	Died
		1753	Marked fever, 4th to 10th days only	Survived
		1768	Severe cattle plague	,,
	1·0	1771	Severe cattle plague	Survived
		1776	,, ,,	,,
		1785	Fever, 5th to 8th days only	,,
	1·5	1790	No symptoms	Survived
		1792	Fever, 5th to 9th days, severe buccal ulceration but no diarrhœa	,,
		1805	Severe cattle plague	Died
	2·0	1808	Severe cattle plague	Died
		1813	,, ,,	Survived
		1815	,, ,,	Died
Lymph glands	0·5	1748	Fever, 5th to 11th days only	Survived
		1764	No symptoms	,,
		1773	,,	,,
	1·0	1781	No symptoms	Survived
		1786	,,	,,
		1787	,,	,,

TABLE XI—(*Continued*).

Tissues.	*Dose gm./100 Kg.*	*Bull No.*	*Symptoms.*	*Result.*
Lymph glands	1·5	1801	Mild indeterminate fever from first day of test. Significance doubtful	Survived
		1806	No symptoms	,,
		1809	,,	,,
	Controls	1777	Severe cattle plague	Died
		1779	,, ,,	,,
		1791	No symptoms	Immune
		1810	Severe cattle plague	Died

It was decided to carry out a further titration, but in consequence of the foregoing results slight modifications in design were introduced. First of all, it was decided to pay no more attention to lung tissue. In addition, it was decided not to titrate thymus separately. The reason for this second decision was the same as that which led to the elimination of the tonsils in the preliminary titration; the tissue is so similar to that of the lymphatic glands that it will give very similar readings, and it is relatively so small that any slight difference—if any—that may exist will not sensibly affect the results. For example, the average weight of the mixed spleens used was about 320 gm. and that of the combined lymphatic glands about 250 gm. Thymuses, after trimming away degenerated tissue, were very small, averaging under 30 gm. It was therefore considered better, in view of the individual variation that had been found to exist in cattle, to concentrate on the titration of spleen and glands only, and to use them on the extra cattle which had been provisionally selected for titrating the other tissues.

Experiment of 27.2.35.

Doses of spleen and lymphatic gland vaccine prepared from six young bulls, ranging from 0·5 gm. to 0·1 gm./100 kg., were given to a number of cattle, six being used for each tissue and each dosage. The 0·5 gm./100 kg. dose was incorporated so as to overlap the dosages used in the first titration, and 0·1 gm./100 kg. was selected as the lowest dose because some of the cattle which had in the earlier test received 0·5 gm. developed mild symptoms, and it was thought that 0·1 gm. could not fail to be below the end-point. The results of the immunity test, carried out seven days after vaccination, are given in Table XII.

It had already been found in the first titration that lymphatic gland tissue produced at least as effective vaccine as spleen. In the second test the vaccine was a sufficiently mixed sample, and a large enough number of test cattle was used to show that gland material is definitely superior to spleen; in fact, the results may be read as confirming Jacotot and Colson's (1934, 1) finding with

TABLE XII.

TITRATION OF GLYCERINATED SPLEEN AND LYMPHATIC GLAND VACCINE. IMMUNITY TEST SEVEN DAYS AFTER VACCINATION.

Tissue.	*Dose gm./100 Kg.*	*Bull No.*	*Symptoms.*	*Result.*
Spleen	0·1	1786	Fever, 3rd to 12th days, severe buccal ulceration, mild diarrhœa	Survived
		1858	Severe cattle plague	Died
		1862	„ „	„
		1864	Fever, 4th to 8th days. Rare buccal vesicles 9th and 10th days	Survived
		1865	Severe cattle plague	„
		1871	Fever, 4th to 11th days. Rare buccal vesicles 10th and 11th days	„
	0·2	1868	Fever, 5th to 11th days, no stomatitis, mild diarrhœa 9th to 11th days	Survived
		1873	Severe cattle plague	Died
		1874	Fever 4th to 11th days, mild buccal ulceration 9th to 11th days, mild diarrhœa 10th to 11th days	Survived
		1875	Severe cattle plague	Died
		1879	„ „	„
		1886	? Thermal reaction	Survived
	0·5	1798	Fever, 5th to 9th days only	Survived
		1800	„ 4th to 9th days only	„
		1802	„ 4th to 11th days only	„
		1889	No symptoms	„
		1890	Fever, 4th to 9th days. Rare buccal vesicles 8th to 11th days	„
		1892	„ 4th to 10th days only	„
Lymph glands	0·1	1797	No symptoms	Survived
		1863	Slight fever 4th to 8th days only	„
		1870	Fever, 4th to 7th days, extensive buccal ulceration 9th to 11th days (healed rapidly), mild diarrhœa 10th to 15th days. Off feed 10th and 11th days only	„
		1876	Fever, 4th to 7th days only	„
		1877	Severe cattle plague	„
		1891	? Thermal reaction	„
	0·2	1872	No symptoms	Survived
		1878	„	„
		1881	Fever, 4th to 7th days	„
		1884	Slight fever, 5th to 7th days (highest morning temperature 102·2°)	„
		1885	Fever, 4th to 7th days	„
		1886	No symptoms	„
	0·5	1765	Fever, 4th to 7th days only	Survived
		1793	„ „ „	„
		1795	No symptoms	„
		1799	„	„
		1818	Slight fever, 4th to 6th days only	„
		1887	No symptoms	„
	Controls	1856	Severe cattle plague	Died
		1857	„ „	Survived
		1859	Died 4th day of tympanites	—
		1860	Severe cattle plague	Died

toluolised vaccine, that it is about two-and-a-half times as good. It may be pointed out, however, that one does not agree with these authors in claiming that glands are only about four times as good as lung, since one found 2 gm./100 kg. of the lung (the highest dose tried) much inferior to even 0·1 gm./100 kg. of gland.

One has in this last experiment to ask oneself whether the end-point of the titration has been reached, and there is little difficulty in answering in the affirmative. So far as spleen is concerned, it certainly has been reached, since deaths occurred with doses of 0·2 gm./100 kg. or less. In the case of glands also, the occurrence of "severe cattle plague," *i.e.*, a response that might as easily have resulted in death as in recovery, in one beast receiving 0·1 gm./100 kg., and quite marked symptoms amounting to a moderate attack of cattle plague in another receiving the same dose, marks this dose as the end-point for all practical purposes. It is to be noted that only the very mildest symptoms developed in cattle which received 0·5 gm./100 kg. of spleen or 0·2 gm./100 kg. of gland.

These results are very encouraging when one recalls the conclusions of other workers in the case of formalinised vaccines. Certainly the latter have not been so fully titrated as one's own glycerinised ones, but the literature does not suggest that doses of this size will be as effective, nor do Jacotot and Colson's titrations of toluolised glycerinised vaccine indicate that it is as good as the plain glycerinised product. Reference has already been made to ostensibly similar titrations carried out by Japanese workers. They do not claim such good results, but their experiments were less comprehensive, and one gathers from their protocols that they did not always use very fresh vaccine. One's own experiments may, in fact, be considered as a supplementary extension of theirs.

The practical implications of these two experiments are best considered in the following general assessment of the whole series of observations, when their correlation with all other results can be discussed.

General Summary.

This paper is confined to studies on glycerinised tissue vaccines against cattle plague, the main sections being concerned with :—

(1) *Safety Interval.*—The interval that must elapse between taking one's virulent material and the safety of the finished product for use.

(2) *Dosage and Immunity Interval.*—These two are studied jointly in order to ascertain whether the size of the dose has any effect.

(3) *Storage.*—In this section one has not only considered the periods over which vaccine can in general be stored, both in cold

storage and at tropical " room temperature," but attempts have been made to determine the features which characterise vaccine deterioration, with the view of compensating or allowing for them if possible in formulating one's vaccination technique in the field.

(4) *Duration of Immunity.*—This has been studied in relation to dosage, in order to ascertain whether the size of dose has any effect on the duration of immunity. Observations have also been made on the phenomena which characterise waning immunity in a vaccinated herd.

(5) *Tissue Titration.*—The smallest dose of various glycerinised tissues that will immunise according to defined standards.

(6) *Practical Applications.*—No critical comparison between glycerinised and formalinised tissue vaccine (or tissues treated with other agents) has been made, but the literature on the latter has been quoted whenever comparison has seemed desirable. Similar references will be made when stating one's conclusions. These conclusions are :—

(1) *Safety Interval.*—Virulent cattle plague tissues treated with twice their weight of 60 per cent. glycerine become avirulent in a period that varies directly with the temperature. On directing particular attention to temperatures of 37° C. and 0° C., it was found that virulence disappeared in three days at the former and about three months at the latter. Small observations on intermediate temperatures afforded intermediate results. The possible criticism– and one that has in fact been actually offered— that the safety interval is uncertain and therefore a drawback, is thus answered. By placing the tissue in an ordinary biological incubator, which every laboratory possesses, the vaccine can be made safe for use in three days, and glycerinised tissue vaccines are thus quite as " safe " as their formalinised, toluolised, etc., counterparts. The slight transient irritation at the site of injection, caused by the presence of glycerine, can be neglected.

(2) *Dosage and Immunity Interval.*—It was considered advisable to study these jointly, since the size of the dose might affect the necessary interval. In the event, such was found not to be the case. Possibly very large scale experiments might show some small balance of evidence in favour of larger doses, but such results would not affect the issue in general practice. The vaccine, virtually regardless of the size of the dose, produces a very serviceable immunity in all cattle, and a solid immunity in most cattle, within three days. After a week it produces a solid immunity in all cattle. This compares very favourably with formalinised vaccine, with which a week seems to be regarded as a minimum interval not always attainable. It must be admitted, however, that formalinised vaccines have not been so thoroughly studied as regards this point.

(3) *Storage.*—Glycerinised vaccine retains its antigenic value when stored in the cold for longer periods than when stored at

higher temperatures. The phenomenon signalising deterioration is failure to immunise a small proportion of vaccinated cattle within the recognised interval. As time progresses, an increasing number of cattle fails to be immunised. It is not possible to compensate for this deterioration to any practical extent by increasing the dose, nor will a significantly higher degree of immunity develop after a slightly longer interval. Such effects are indeed appreciable in some small degree, but any hope of benefiting from them in practice is vetoed by great individual variation in the cattle. If a vaccine is to produce solid immunity in all cattle, the limits of storage are not much more than one month at 37° C. or one year at 0° C. If, however, " outbreak stopping " value only is at issue, these periods could safely be doubled. It is not possible to decide how these findings compare with formalinised vaccine. In any case the storage properties of glycerinised vaccine make it very suitable for Sudan conditions.

(4) *Duration of Immunity.*—No period can be definitely stated owing to great individual variation in cattle. However, in a *herd* vaccinated with fresh vaccine, immunity in a small proportion of individuals will begin to wane after about eight or ten months, at which period they may on exposure to infection develop symptoms; an odd beast may even die. And so on, with advancing time. Where the safety of *herds*, however, is at issue, the persistence of solid immunity in so large a proportion of individuals at ten months' interval indicates that a herd as a whole will be fairly safe for at least a year after vaccination. The size of the dose has within reasonable limits no influence of practical value on the duration of immunity, although there is some slight benefit to be derived from very large doses.

(5) *Tissue Titration.*—Having found that the size of the dose is a factor having relatively minor influence, the minimum dose of all useful tissues is of major importance. It has been shown that the other lymphoid tissues are at least as good as spleen, but that lung tissue, which is constantly used by some workers, is of such low value that it is probably not worth the trouble of incorporating. Titrations carried approximately to the end-point showed that none of the useful tissues could be relied upon to produce a solid immunity in seven days with a lower dose than 1 gm./100 kg. Although the production of solid immunity has been one's arbitary standard throughout the whole series of experiments, it is not possible to assess the relative antigenic value of useful tissues without relaxing it when dealing with the very lowest doses. This relaxation is justified by the knowledge that the size of the dose has some small influence on the rate of immunity production. Having found thymus at least as good as spleen, attention was concentrated on spleen and lymphatic glands because they yield about 90 per cent. of available tissue.

A serviceable immunity, short of solid immunity, was not produced by less than 0·5 gm./100 kg. of spleen, or by less than 0·2 gm./100 kg. of lymphatic gland, although a large proportion of cattle were adequately protected by still lower doses. These results do not differ materially from those of the Japanese workers when full consideration is given to their conditions of experimentation. Whatever standard of immunity one adopts, however, it seems that glycerinised tissues protect in rather lower doses than formalinised tissues, even if the most favourable conclusions regarding the latter are taken as a basis for comparison. It is admitted, however, that critical parallel tests have not been carried out. The significance of these titrations will be considered in the following discussion on the practical applications of one's results as a whole.

(6) *Practical Applications.*—These are most conveniently considered in chronological order starting with the production of the vaccine, and proceeding through the stages of storage and issue to its administration in the field and the results expected from it.

The vaccine is easily prepared, using the spleen, lymphatic glands, tonsils and thymus of virus producers employed in serum production. Preliminary passage through a domestic meat mincer allows the tissue to be at once mixed with double its weight of boiled 60 per cent. glycerine. After a few days' soaking in this state, it is found that the tissue can be fairly easily ground to a paste that will pass through an inoculating needle of moderate calibre by an ordinary paint mill. A small residue, consisting almost entirely of connective tissue, is rejected by the grinding faces and can be discarded. If the vaccine is to be used within a week or two it need not be placed in cold storage, otherwise this procedure is necessary. In the Sudan, tissues are minced and treated with glycerine at the serum laboratory at Malakal and the product is placed in cold storage. Electricity is not available, but it has been found that the recently introduced oil-operated refrigerators are extremely efficient. Periodically, consignments are sent to Khartoum, also in cold storage, by Nile steamer, for further treatment and storage. Vaccine is produced much more quickly than it can be used during the six or seven months while the Malakal laboratory is working at top pressure, and a large reserve stock is accumulated at Khartoum for issue while the former laboratory is closed.

The stock in Khartoum is not necessarily immediately incubated and ground, for which there are two reasons. First, there is no point in incubating at 37° C. for three days unless the vaccine is to be issued fairly soon. It has been shown that antigenic value is better conserved in cold storage, and that even in cold storage the virulence will disappear in about three months. Therefore, by maintaining vaccine destined for issue in the rela-

tively distant future at low temperature some months are added to its " life." Vaccine needed for early issue is incubated and reground as and when necessary. The second reason for not dealing with the crude vaccine earlier than necessary is that the longer it is left soaking in glycerine the easier it becomes to pass it through a paint mill, since the lymphoid tissue becomes completely macerated and the machine rejects the connective tissue more readily. The question of storage was originally investigated in order to carry stocks for about six months. As has been shown, they can be carried for a year more.

The next point for discussion is the dose, which has to be determined in consideration of what it is to accomplish. It has already been shown that for practical purposes the size of the dose has a negligible bearing, both on the rate of immunity production and on the duration of immunity. Quality rather than quantity of vaccine is the prime factor. Again, it has been shown that whereas a dose of 1 gm./100 kg. of vaccine stored for at least a year in cold storage or at least a month at 37° C. will produce solid immunity in a week, nevertheless no smaller dose of any tissue will accomplish this even with the freshest sample. It is therefore obvious that if solid immunity is to be the aim no smaller dose than 1 gm./100 kg. of mixed tissue vaccine should be issued. The serviceable immunity produced by 0·5 gm./100 kg. of spleen, and the superiority of lymphatic glands over spleen in producing serviceable immunity in smaller doses, may be regarded as some kind of indeterminate reserve, valuable no doubt, but incapable of precise assessment. When dealing with cattle which *must* be solidly immunised, *e.g.*, working oxen or cattle moving along trade routes, this reserve will give the operator confidence that the stock vaccine will achieve its object. When called upon to vaccinate a threatened nomad herd with a shortage of vaccine, this reserve may be used to reduce the dosage slightly with the knowledge that casualties will be minimal. Furthermore, by using the 1 gm./100 kg. dose, which was the one used in studying the duration of immunity, it can be expected that all cattle moving along trade routes will be solidly immune for at least as long as is necessary, and nomad herds should be for practical purposes " immune " for at least a year. The policy in the Sudan, therefore, is to carry heavy stocks in cold storage in Khartoum, supplementing them locally when necessary. Sufficient stock for almost any emergency can be maintained. The dose laid down for field use is 1 gm./100 kg. (translated, however, into terms of volume of vaccine for the sake of native inoculators). When used on working oxen or cattle moving along regular trade routes the vaccine is given as soon as possible after issue—usually within a week—although it could safely be stored for a month. The maximum permitted period for storage pending use on nomad-owned cattle is laid down as two months.

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BORDER PINING IN SHEEP—II.

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Introduction.

An investigation of Northumbrian or Border "Pining"[1] has indicated that parasitic gastro-enteritis is ultimately responsible for the main features of the disease. The numbers of nematode parasites present in typical cases showed great variation, being greatest in cases examined during the summer months (Table 1A), when the common form of pining occurs, and fewest in chronic, lingering cases which died or were killed *in extremis* during the following winter (Table 1B).

It is to be remarked that what may be termed acute cases of pining (the common type) occur in summer when relatively the pasture is at a high nutritive level. This fact does not at the outset suggest a connection with faulty nutrition, and in all probability it is due to the more rapid multiplication of the worms during the warmer summer months. There is also the possibility, however, that some deficiency in pining pastures, even when at their best, renders the sheep more vulnerable to parasitic attack. Under-nutrition may render sheep less resistant to the establishment of heavy worm infestations and may thus, in part, account for the very high worm burdens in the acute cases recorded in Table 1A. In addition, faulty nutrition is thought to render the host more susceptible to the harmful effects of an established infestation.

There is considerable evidence in support of the former view that general under-nutrition does lower the resistance of the vertebrate host to attack by parasitic worms. Thus Chandler[2] found that when white rats were kept on an inadequate diet of coarse vegetables, on which they became emaciated, more of the nematode worms *N. muris* were able to establish themselves from a given inoculation of infective larvæ than when the rats were adequately fed. In addition, the worms were harboured for a longer period.

Fraser and Robertson[3] fed two groups of three-months-old worm-free lambs, for a month prior to turning them out on to infected pasture, on 7 to 8 lb. a day of green tares and bruised oats, the lambs in one group (controls) receiving in addition three pints of separated milk each and 1 lb. of mixed meals, daily. The two groups were then kept on the same pasture, heavily infected with *H. contortus*, for 46 days, the meal supplement, but not the milk, being continued to the controls. At the end of this period *post-mortem* examinations were carried out on the entire

TABLE 1A.

POST-MORTEMS DURING SUMMER MONTHS. ACUTE PINING.

Date.	Reason for P.M.	Blood Picture before P.M.			Condition of Mucous Membrane of Abomasum.	Egg Count.	Worms in Abomasum.	Worms in Small Intestine.	Worms in Large Intestine.	Diagnosis.
		R.B.C. (Count).	R.B.C. (Vol.)	Hb. % of Normal.						
23.6.33 (4 yrs.)	Died	4·85	15	38	Thickened, roughened and congested	3,700	60,000 : chiefly *Ostertagia circumcincta* and *Hæmonchus contortus*	Not counted. Numerous. *H. Contortus* and *Nematodirus filicollis*. About 10 yds. of tapeworm	Many *Chabertia ovina*	Parasitic gastro-enteritis.
27.7.34 (5 yrs.)	Killed, in weak state	6·65	15	45	Thickened, roughened and like grained leather	1,600	38,600 : chiefly *O. circumcincta, H. contortus* and *Cooperia oncophora*	8,200 : chiefly *C. oncophora, H. contortus* and *Moniezia expansa*	*Trichuris ovis* and *Ch. ovina* present	Parasitic gastritis.
2.8.33 (4 yrs.)	Killed as in weak state	6·17	22	59	Thickened and roughened	300	Not counted Heavy infestation of *O. circumcincta, O. trifurcata* and *N. filicollis*	Not counted No tapeworms	*Ch. ovina* and *T. ovis* present	Parasitic gastritis.
24.8.33 (4 yrs.)	Killed in weak state	4·78	14	40	Thickened and like grained leather	1,000	21,500 : chiefly *O. circumcincta, O. trifurcata, Trichstrongylus extenuatus* and *H. contortus*	14,000 : N. *filicollis, Cooperia curticei, Trichostrongylus colubriformis* and *Trichostrongylus vitrinus*	*Ch. ovina* present	Parasitic gastro-enteritis.
20.7.34 (2 yrs.)	Died	7·50	21	58	Like grained leather	5,200	17,000 : chiefly *O. circumcincta*	26,000 : chiefly *C. oncophora, T. extenuatus* and *M. expansa*	*Ch. ovina* present	Parasitic gastro-enteritis and Parasitic broncho-pneumonia (many lung worms present).
28.7.34 (5 yrs.)	Died	5·60	—	52	Thickened, roughened and like grained leather	8,000	47,200 : chiefly *O. circumcincta, T. colubriformis* and few *H. contortus*	15,300 : chiefly *N. filicollis* and *C. oncophora*	Many *Ch. ovina* present	Parasitic gastro-enteritis and Parasitic broncho-pneumonia (lung worms present).

Key—
R.B.C. Count represents millions per cu. mm.
„ Vol. represents % Vol. of red blood cells.
Hb expressed as % Normal takes 11·45 gms. % as 100.

flock. It was found that the poorly-fed group of lambs averaged only 60 lb. live-weight and harboured an average of 103 worms, whereas the better fed (control) group averaged 84 lb. live-weight and harboured only an average of 31 worms.

Taylor[4] records experiments in which the rate of infection was controlled by giving the animals in experimental and control groups equal doses of infective larvæ and he concludes that undernutrition of the host results in inability to acquire an immunity to parasitic infestation. Six worm-free lambs, about eight months old, were divided into three groups of two each, one group being fed on hay and cake, another on hay alone, and the third on straw only. All lambs received 40 doses of 500 infective larvæ (mixed *Trichostrongyles*) spread over a period from the end of October until the beginning of January. By the following March the worm egg counts had fallen respectively to 300 and 800 per gramme fæces in the two hay and cake fed lambs, whereas it reached 2,850 in one of the lambs fed on straw. On *post-mortem* examination the numbers of worms found in the lambs in the former group were 348 and 6,468, whereas in the latter the numbers were 11,800 and 13,900. The lambs fed on hay alone occupied an intermediate position as regards worm burden, 8,778 and 9,800 worms being found. In a further experiment six lambs were divided into two groups, one of which received hay and cake, whilst the other had hay only. Here Taylor came to a similar conclusion. All six lambs were known to be fairly heavily infected at the commencement of the experiment, but the animals were kept under conditions which precluded their reinfection. In the well-fed group the egg counts fell steadily from December until the following August, whereas in the poorly-fed group they rose enormously. Since no further worms were picked up it was concluded that those already present were able to produce many more eggs when the host was underfed.

There is likewise considerable evidence that under conditions of malnutrition, in which individual essential food factors such as minerals and vitamins are deficient, a lowered resistance to helminthic infestations results. Foster and Cort[5] found that dogs which, by repeated infection, had acquired an immunity to hookworms lost this immunity after feeding on a diet deficient in both minerals and vitamins, the worm egg count rising rapidly. This was apparently due to the worms already present being able to produce more eggs, since further infection with fresh worms was impossible under the prevailing conditions. On returning to a full diet there was a rapid fall in the egg count. The authors conclude the existence in the well-fed animal of a mechanism inhibiting maturation and egg production of the worms. In a further and more striking experiment by the same authors (Foster and Cort[5]) two dogs with egg counts nil were kept on a good diet, and when given doses of infective larvæ were found to be very

resistant to infection, the egg counts remaining nil. These dogs were then kept on a deficient diet for 90 days and were given doses of 500 infective larvæ (*Ancylostoma caninum*) every fortnight. The egg counts rose rapidly, but on returning to the adequate diet, the doses of larvæ still being continued, the egg counts fell and worms were eliminated.

Deficiency of vitamins alone is thought to lower resistance to worm infections. Thus Hiraishi,[6] working with Ascariasis in young pigs, Ackert and Crawford[7] investigating parasitism in chickens and Nagoya[8a and b] testing the resistance of dogs and white mice to *Ancylostoma caninum*, all found that feeding on a vitamin A deficient diet lowered the resistance to worm infections. Similarly, deficiency of vitamin B was found by Ackert and Nolf[9] to lower the resistance of puppies to *A. caninum*. Ackert and Spindler[10] found lowered resistance to parasitism in chickens fed on a vitamin D deficient diet.

Deficiency of mineral elements alone may also render the host less resistant to helminthiasis, although this has not, as yet, been established with certainty. Clapham[11] found that chicks after four weeks on a diet deficient in calcium and phosphorus, but containing adequate vitamins, had developed, 24 days after being given 200 eggs of *Heterakis gallinæ*, a heavier infestation than control birds receiving calcium carbonate and phosphate in addition to the deficient diet. Statistical reports, also, from all parts of the world, indicate that in phosphorus-deficient areas deaths from causes other than actual phosphorus deficiency are higher than in areas where there is no deficiency (Theiler and Green[12]).

The *post-mortems* carried out in the winter on seven cases of chronic pining (Table 1B) revealed comparatively light worm burdens, the numbers in the abomasum varying from 4,000 to 12,000 per case. Equally heavy worm burdens have frequently been found in fat ewes at the abattoir, and in the opinion of other workers sheep may harbour 10,000 strongyloid parasites without showing clinical symptoms of parasitic disease. The failure of these seven ewes to recover condition, although the worm burden was at this time only slight, suggests that under-nutrition may not only be indirectly concerned in the production of acute pining, by lowering resistance to worm parasites, but may also be more directly responsible for the continued ill-health seen in the chronic cases.

Defective nutrition might result, on the one hand, from imperfect utilisation of food by the sheep due to permanent injury of the gastric mucosa when the worm infestation was at its height the previous summer, although the pasture in itself might be adequate. On the other hand, the pastures on pining farms might be deficient either as regards Net Digestible Energy or as regards minerals, particularly in winter.

Date.	Reason for P.M.	Blood Picture before P.M.			Condition of Mucous Membrane of Abomasum.	Egg Count.	Worms in Abomasum.	Worms in Small Intestine.	Worms in Large Intestine.	Diagnosis.
		R.B.C. (Count).	R.B.C. (Vol.)	Hb. % of Normal.						
19.11.33 (6 yrs.)	Killed, in weak state	8·43	26	69	Thickened and roughened No congestion Studded with small raised plaque-like areas, like healed scars	700	Not counted. Chiefly *O. circumcincta, O. trifurcata* and *H. contortus*	Not counted. Tapeworms present	—	Parasitic gastritis. Might have recovered with better nutrition
19.12.34 (4 yrs.)	Killed, in weak state	7·06	18	64	Thickened, roughened and slight congestion	1,400	5,700 : chiefly *O. circumcincta*, also *C. oncophora* and a very few *H. contortus*	4,000 : chiefly *C. oncophora, O. circumcincta*, and *M. expansa*	*T. ovis* and *Ch. ovina* present	Under-nutrition coupled with slight Parasitic gastro-enteritis
17.2.34 (5 yrs.)	Died	10·53	27	91	Slightly thickened	500	12,500 : chiefly *O. circumcincta*. A few *H. contortus*	2,400 : chiefly *T. extenuatus*	*Ch. ovina* present	Broncho-pneumonia probably assisted by Parasitic gastro-enteritis.
7.3.34 (5 yrs.)	Died	5·21	20	48	Slightly roughened and inflamed	5,100	10,500 : chiefly *O. circumcincta*. A few *H. contortus*	Not counted *Bunostomum trigonocephalum, C. oncophora* and *N. filicollis* present	Many *Ch. ovina* present	Broncho-pneumonia. Probably parasitic gastro-enteritis contributory.
18.12.34 (3 yrs.)	Killed, in weak state	11·90	33	102	Very slightly thickened and roughened, but many healed scars	1,300	5,000 : *O. circumcincta*	4,600 : chiefly *Cooperia* species and *N. filicollis*	Many *T. ovis* and *Ch. ovina* present	Under-nutrition coupled with slight Parasitic gastro-enteritis and Broncho-pneumonia.
18.12.34 (6 yrs.)	Killed, in weak state	12·92	36	108	Thickened, roughened and like grained leather	8,200	6,900 : chiefly *O. circumcincta*	900 : *C. oncophora* and *N. filicollis*	*T. ovis* and *Ch ovina* present	Under-nutrition and slight parasitic gastritis.
18.12.34 (6 yrs.)	Killed, in weak state	9·16	28	80	—	4,700	3,900 : *O. circumcincta*	2,200 : chiefly *C. oncophora*	*T. ovis* and *Ch. ovina* present	Under-nutrition (enlarged molar teeth probably contributory) and slight parasitic gastro-enteritis.

As a result of practical experience flockmasters believe that under-nutrition is in some manner concerned in the causation of pining. Thus, all are aware of the benefit of temporary changes to pastures of a different type, and for generations this has been adopted both for treatment and prevention. Moreover " pining " farms are unsuitable for growing sheep, which do better if wintered from home. It is also found necessary to delay mating the young Cheviot ewes until they are nearly three years old. It is customary to take four crops of lambs from these ewes, after which, on account of waning vigour, they are drafted to lower-lying farms, the average life of ewes on pining farms, allowing for wintering away as hoggs, thus being approximately five years. At this time many of these ewes are showing clinical symptoms of pining, but it can be stated that the great majority recover completely after transference to the richer pastures and more favourable conditions. This disposes of the suggestion that an attack of pining causes serious permanent injury to the gastric mucosa, and more weight is therefore given to the view that the pastures themselves may be nutritionally inadequate.

The present investigation into the cause of pining was undertaken from three aspects. The metacarpal bones of three sheep suffering from chronic pining were examined and compared with similar bones from fat sheep of the same breed, obtained at the abattoir, in order to see whether they reflected possible nutritional disturbances. The gastric mucosa from three pining sheep was examined microscopically in serial sections, and compared with the normal structure. In addition, samples of pasture from hill farms where pining occurs were analysed in an endeavour to assess the general nutritive value of the grazing and to ascertain if any important mineral elements were deficient.

Experimental Section.

Examination of Bones.

Photographs are reproduced (p. 56) of two pairs of metacarpal bones split longitudinally. Bones 1A and 1B are from a normal and a pining Blackface sheep respectively. Bones 2A and 2B are from a normal and a pining Cheviot sheep respectively, and these latter have been dried in an air oven before being photographed. As shown in Table 2 the bones from the pining sheep are not much shorter than those of the normals, 5, 6, 7, 8, 9 and 10, but they are definitely thinner both in shaft diameter and more particularly in the thickness of the corticalis. In the pining bones less marrow was present and it had the appearance of gelatinous degeneration. It is not unreasonable to associate these differences with differences in nutrition, various workers having found in many different species that gelatinous degeneration of the bone marrow results from inanition.[13] In Table 3 it will also be noted that the blood calcium ranges from 7·20 to 8·37 mgs. per cent.

serum as compared with the commonly accepted normal of about 10 mgs. per cent. Attention has already been drawn[1] to this feature of pining.

TABLE II.

Comparison of Metacarpal Bones of Normal and Pining Sheep.

No.	*Breed.*	*Condition.*	*Length of Metacarpal (cms.).*	*Fresh Wgt. Metacarpal (gms.).*	*Thickness Shaft Wall (mms.).**	*Thickness of Shaft / Length.*	*Fresh Wgt. / Length.*
1	Cheviot	Pining	10·4	17·5	3·4	0·33	1·68
2	,,	,,	11·2	18·8	3·3	0·29	1·68
3	,,	,,	10·7	16·3	2·9	0·27	1·53
4	Blackface	,,	9·6	14·0	2·6	0·27	1·45
					Mean	0·29	1·58
5	Cheviot	Normal	11·2	32·7	4·5	0·40	2·92
6	,,	,,	12·2	37·0	4·5	0·37	3·03
7	,,	,,	11·7	32·7	4·1	0·35	2·79
8	Blackface	,,	10·6	26·3	4·0	0·38	2·48
9	,,	,,	11·5	29·3	4·9	0·43	2·54
10	,,	,,	11·6	31·7	4·7	0·41	2·73
					Mean	0·39	2·75

*Given as the sum of both sides of the shaft wall as seen in longitudinal section.

TABLE III.

Blood Picture, Worm Count and Worm Egg Count of Pining Ewes prior to Post-mortem and Examination of Metacarpal Bones.

No.	*Breed.*	*Ca % in Serum.*	*Inorg. P % in Whole Blood.*	*R.B.C. Count.*	*Hb% Gms.*	*Hb% Normal.*	*White Blood Cells.*	*No. Worms in Abomasum.*	*No. Worms in Small Intestine.*	*Egg Count.*
1	Cheviot	8·15	11·68	11·90	11·7	102	9,400	4,900	4,600	800
2	,,	7·96	5·84	9·16	9·7	85	7,400	3,900	2,200	4,700
3	,,	7·20	4·92	3·92	3·8	33	4,600	11,800	2,600	19,200
4	Blackface	8·37	6·01	7·29	7·3	64	5,200	5,700	3,900	1,400

Examination of Gastric Mucosa.

In all the pining cases examined the mucous membrane of the fourth stomach was thickened and roughened in a manner not noted in healthy abattoir sheep. The thickening occurred in areas both on the abomasal folds and in the pyloric region, and was doubtless the result of irritation from strongyloid parasites. These not only pierce the mucosa in order to abstract nourishment but larval forms of certain species (*O. circumcincta*) invade the mucosa and there develop. The thickened mucosa macroscopically resembles grained leather. In an endeavour to study the minute

changes leading to this alteration in the mucosa and to look for evidence of permanent injury, specimens of tissue were obtained from pining cases, and after fixation were embedded in paraffin

LARGE METACARPAL BONES OF PINING AND NORMAL SHEEP.

Before Drying. After Drying.

1A Pining Blackface. 1B Normal Blackface. 2A Pining Cheviot. 2B Normal Cheviot.

and cut into serial sections with a Leitz microtome. The sections were stained with hæmatoxylin and eosin, and with van Gieson which acts specifically on fibrous tissue. Specimens from four pining sheep were examined and compared with the normal.

The thickening of the stomach wall appeared to be due to proliferation of cells in the mucous membrane, and extended no deeper than the muscularis mucosa. There were no signs of active inflammation, the changes having proceeded to the stage of epithelial proliferation. Careful search of sections using the high powered objective revealed many larvæ, presumably of trichostrongyle parasites, measuring up to three millimetres in length and lying coiled within the mucosa. They were not encapsulated. In one or two cases larvæ appeared to be making their way to the surface of the tissue. The thickening of the tissue was due to cellular proliferation with subsequent degeneration and no newly-formed fibrous tissue was detected.

Grass Analyses.

Pastures were analysed from three different farms where pining occurs. Small plots were fenced off and samples of grass cut in July and October, 1934, and in January, 1935. On each of the farms one plot was laid out on the best pasture, and one or more on the poorest pasture where pining was believed to be most prevalent. The " best " pastures were usually enclosed fields (Farms No. 1 and No. 2), but on Farm No. 3 the " best " pasture was on the southerly aspect of a well-known pining hill. In taking the samples the coarsest tufts were discarded and an attempt was made to select herbage representative of that eaten by the sheep. Although the samples do not necessarily reflect the selective grazing of the sheep, it is believed that they are fairly representative of the state of the pasture at the time. The approximate analyses of the samples are given in Table 4 on a dry matter basis. The Starch Equivalents (Net Digestible Energy) of the pastures, indicated in this table are calculated on the assumption that the digestibilities of the individual constituents were approximately the same as for grasses or hays quoted in the Ministry of Agriculture's Bulletin 48,[14] which contained, firstly, about the same percentage of fibre, and secondly, a similar percentage of protein. Kellner's[15] method of calculating starch equivalents from the digestible food constituents was employed, assuming " V " numbers corresponding to those grasses already selected for comparison from the Ministry of Agriculture's Bulletin.

The figures thus obtained for the hill pastures are obviously only approximate, but unless lengthy metabolism experiments are carried out this seems the only method available. Such computations should reveal any gross nutritive differences in pastures, although small differences cannot be regarded as significant. They are probably more helpful than the calculations of " caloric values " occasionally employed[16] since they represent net energy available for production rather than total digestible calories.

Estimations of the total ash, CaO, P_2O_5 and Fe were carried out and the results are expressed in Table 3 as percentages on

the dried grass, iron being also stated on the ash for comparison with published data.

The calcium estimation was carried out on the hydrochloric acid extract of the ash, using Kramer and Tisdall's[17] modification of the Halverson-Bergeim[18] micro-technique. The solutions were neutralised to brom cresol green, the calcium oxalate being precipitated at a *p*H of about 5. The phosphorus was estimated colorimetrically on the neutralised hydrochloric acid extract of the ash by an adaptation of Briggs[19] modification of the Bell Doisy[20] micro-technique. The iron was estimated colorimetrically on the neutralised hydrochloric acid extract of the ash by an adaptation of Wong's[21] method for the determination of iron in blood.

Discussion on Grass Analyses.

From Table 4 it will be noted that the differences in starch equivalents of the different pasture areas on the three pining farms merely reflect the previously expressed opinions of flockmasters, formed on the basis of experience. The pastures described as " best " have higher starch equivalents than those considered as the " worst pining hills " but the difference is not so striking as to suggest gross differences in energy value. As would be expected, the January figures are generally lower than the July figures, but even the lowest values, 3 (*b*) and 3 (*c*), would be adequate for maintenance so long as the sheep succeed in obtaining sufficient quantity. On these rough hill pastures, however, the readily eaten herbage is relatively sparse, and greater distances, with proportionately greater energy expenditure, must be covered to secure an adequate daily intake. It seems quite probable that the failure of pining sheep to recover condition when the worm burden falls off in winter may be due to difficulty in securing a sufficient quantity of nutritious grass. The protein content considered in relation to the stage of growth of the grass as sampled is suggested as low, but the values, 13·1 per cent. for the nine best samples and 10·3 per cent. for the twelve worst, cannot be regarded as indicating relative protein deficiency.

When, however, the mineral content of the samples is considered there are more definite indications of deficiency. From Table 5 it will be seen that, with the exception of the best pasture on Farm No. 3, the calcium figures are all low by comparison with pastures generally recognised as adequate. For pastures No. 3 (*b*) and (*c*), and No. 1 (*b*), the figures range from 0·24 per cent. CaO down to the phenomenally low value of 0·09 per cent. Orr's average figure[22] for British cultivated pastures is over 1 per cent., and even for " poor hill pastures—partly grazed " is as high as 0·56 per cent., *i.e.*, more than twice the value of some of these pining pastures, which approximate rather to the calcium-low grazing of the Falkland Islands and the Island of Lewis. The lowest figure quoted in Orr's monograph, 0·29 per cent. for

the Island of Lewis, is still considerably higher than the three pining hills referred to, and the average for No. 3 (*b*) is only 0·16 per cent.

TABLE IV.

THE NUTRITIVE VALUE OF PASTURES ON PINING FARMS. EXPRESSED AS STARCH EQUIVALENT. (CALCULATED ON DRY MATTER BASIS.)

Farm. No.	*Pas-ture.*	*Description.*	*Month.*	*Crude Fibre.*	*Crude Pro-tein.*	*Crude Ether Extract.*	*Soluble Carbo-Hydrate.*	*Starch Equiva-lent.*	*Aver-age, S.E.*
1	(*a*)	Best pasture. Thought that sheep never pine here	July	25·0	14·0	3·1	49·9	50	
			Oct.	24·9	13·3	2·5	48·9	49	48
			Jan.	27·5	12·0	2·2	49·2	45	
	(*b*)	Pining hill	July	29·2	11·7	1·9	50·3	45	
			Oct.	28·5	9·4	2·0	50·9	46	43
			Jan.	31·0	7·5	1·8	51·9	37	
2	(*a*)	Best pasture	July	26·1	13·0	2·9	51·5	53	
			Oct.	24·3	14·2	2·7	50·1	49	51
			Jan.	27·7	13·8	1·8	49·2	52	
	(*b*)	Pining hill for years	July	27·4	9·3	2·4	53·9	43	
			Oct.	26·6	14·9	2·6	47·5	51	45
			Jan.	30·1	9·6	1·9	50·6	42	
3	(*a*)	Best hill Southerly aspect of pining hill	July	25·8	11·8	2·8	52·2	53	
			Oct.	25·0	13·1	2·5	50·8	50	49
			Jan.	28·0	12·4	2·0	49·0	45	
	(*b*)	Pining hill	July	29·4	12·2	2·6	50·2	46	
			Oct.	28·6	9·7	1·9	51·9	46	43
			Jan.	31·8	6·8	1·6	51·5	36	
	(*c*)	Pining hill Same as (*b*) but lower down	July	29·3	10·2	2·0	51·0	45	
			Oct.	29·1	14·4	2·1	45·1	44	42
			Jan.	30·4	7·6	1·9	50·7	36	

With regard to phosphorus the figures are perhaps less striking, although they compare unfavourably with average British pastures and in some cases might well be incriminated as deficient. No. 3 (*b*) of Table 5 shows an average value of 0·34 per cent. P_2O_5 in contrast to the average value of 0·61 per cent. given by Orr[22] even for "poor hill pasture—partly grazed," while the January figure of 0·25 per cent., considered in the light of the corresponding starch equivalent, begins to approach the values at which specific aphosphorosis has been recorded in other parts of the world.[23]

TABLE V.

MINERAL CONTENT OF PASTURES ON PINING FARMS.

Farm No.	*Pasture.*	*Description.*	*Month.*	*Total % Ash.*	*CaO %*	*P_2O_5 %*	*Fe %*	*Fe % Ash.*
				Percentages of Dried Grass.				
1	(*a*)	Best pasture Thought that sheep never pine	July	8·01	0·39	0·67	0·014	0·17
			Oct.	10·36	0·42	0·73	0·015	0·14
			Jan.	9·12	0·36	0·46	0·016	0·17
	(*b*)	Pining hill	July	6·79	0·19	0·53	0·013	0·19
			Oct.	9·18	0·22	0·43	0·011	0·12
			Jan.	7·71	0·20	0·28	0·016	0·21
2	(*a*)	Best pasture	July	6·50	0·37	0·72	0·015	0·23
			Oct.	8·74	0·52	0·72	0·014	0·16
			Jan.	7·46	0·31	0·64	0·060	0·80
	(*b*)	Pining hill for years	July	6·96	0·41	0·30	0·019	0·27
			Oct.	8·41	0·44	0·35	0·021	0·25
			Jan.	7·78	0·31	0·35	0·038	0·49
3	(*a*)	Best hill, but pining	July	7·36	0·75	0·39	0·027	0·37
			Oct.	8·54	0·80	0·40	0·027	0·32
			Jan.	8·57	0·45	0·43	0·060	0·70
	(*b*)	Pining hill	July	5·60	0·16	0·44	0·014	0·25
			Oct.	7·87	0·22	0·34	0·028	0·36
			Jan.	8·30	0·09	0·25	0·022	0·26
	(*c*)	Pining hill, same as (*b*) but lower down	July	7·58	0·18	0·51	0·010	0·13
			Oct.	9·31	0·24	0·36	0·012	0·13
			Jan.	9·37	0·14	0·31	0·025	0·27

It is interesting to note that this sample is also the one lowest in calcium content, but that there is no general parallelism between calcium and phosphorus in Table 5. Farm No. 3 (*a*) shows the highest calcium associated with a phosphorus content not dissimilar to that of Farms No. 1 (*b*), 3 (*b*) and 3 (*c*), which are characteristically low in calcium, while Farm No. 2 (*a*) which shows the highest phosphorus shows a calcium content considerably lower than No. 3 (*a*).

With respect to iron it is difficult to assess the significance of the analytical data given in Table 5, although with the exception of Farm No. 3 (*a*) and the unexpectedly high January samples of No. 2 (*a*) and 2 (*b*), which may possibly have suffered soil contamination, the figures are lower than Orr's average of 0·33 per cent. Fe

on ash for 30 samples of Scotch hill pastures, and many of them are lower than the upper limit (0·22 per cent.) of areas in New Zealand where " bush sickness " occurs, a disease attributed by Aston to iron deficiency. Pining hill No. 3 (*c*) shows only 0·13 per cent. Fe on the ash (0·01 per cent. on the dry matter of the grass) and the temptation would be to incriminate this as a contributory cause of " pining " (as it is in the case of the Scottish " pine ") but for the fact that on No. 1 (*a*), on which pining apparently did not occur, the iron values are equally low. Further investigation of low iron as a possible factor in the ætiology of the disease is necessary. Provisionally it would appear that all three elements, calcium, phosphorus and iron, may be unusually low in the herbage of pining farms, but that even on adjoining hills the type of mineral deficiency may vary. One element only may be low, or all three may be deficient, as in the case of pining hills 3 (*b*) and 3 (*c*).

The lighter skeletal structure of the pining as compared with the normal sheep of similar breed, as shown in Table 2, and the low blood calcium shown in Table 3, may possibly be correlated with the low mineral content of the pastures shown above.

General Conclusions.

(1) Analyses of pastures on three farms in Northumberland on which " pining " in sheep occurs suggest deficiency in calcium, phosphorus and iron, one or all three minerals being involved in varying degrees and possibly inducing specific malnutrition. The net energy value of the grazing is sufficient for maintenance if the sheep succeed in obtaining an adequate daily intake, but the sparse distribution of readily eaten herbage may possibly be responsible for general under-nutrition at certain times of the year.

(2) Nematode parasites, belonging chiefly to the sub-family *Trichostrongylineæ*, have been shown to be the apparent cause of the main symptoms of acute pining, but the numbers of parasites present during the winter months are insufficient to account for the persistent anæmia and emaciation characteristic of chronic pining.

(3) It is suggested that Border Pining is due to a combination or interaction of malnutrition and gastro-intestinal parasitic infestation.

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TRANSMISSION EXPERIMENTS WITH BOVINE MALIGNANT CATARRH.

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INTRODUCTION.

Although malignant catarrh of cattle has undoubtedly been recognised as a clinical entity in Europe for more than a hundred years, modern investigation of the disease, at least in Germany, may be said to have been stimulated by Dobberstein's observation (1925) that cattle dying from malignant catarrh show a characteristic infiltrative lesion in the brain. Dobberstein's findings were confirmed by Glamser (1926), and Goetze and Liess in 1929 carried the work a stage further when they reported successful transmission of the disease by blood-inoculation to three cattle out of seven inoculated. Attempts to transmit with brain, aqueous humour and lymphatic gland failed ; and Goetze and Liess assumed that in all probability natural transmission is effected through some blood-sucking vector.

Meanwhile, in South Africa, Mettam (1923) had published a description of " snotsiekte," which he differentiated from malignant catarrh, largely because he was able to transmit " snotsiekte " to cattle by the injection of fairly large quantities of fresh blood. Mettam drew attention also to the constant swelling of the lymphatic glands, both in natural and experimental cases of " snotsiekte," which he regarded as further proof of the distinctness of the two diseases.

While the investigations of Goetze and Liess were in progress, Quinlan, who was in Hannover at the time, drew their attention to the similarity between Mettam's findings in " snotsiekte " and the results they were obtaining in their transmission experiments with malignant catarrh. After an examination of Mettam's paper Goetze and Liess concluded that there was nothing upon which a separation of the two diseases could be based. In subsequent papers the same authors (1930) and Goetze (1930) continued to record the progress of their investigations. From observations made on more than 40 cases Goetze (1930) compiled a most valuable clinical account of the disease, which he says may take four forms : the per-acute form, the alimentary form, the head-and-eye form, and the mild form. The head and eye form is the best known.

Summarising their findings over a number of years, Goetze (1930), stated that blood transfusion from diseased cattle had given a positive result in 40 per cent. of cases (13 out of 34) after an incubation period of from 16 days to 10 months, and that the disease had been carried through four serial passages in cattle. Filtration experiments or inoculations with centrifuged blood had given only negative results. Blood several hours or a day old was no longer active. Goetze and Liess failed to transmit the disease with blood and other tissues to mice, rats, guinea-pigs, cats, dogs and pigs ; in goats they believed that they had succeeded in producing a mild form of the disease. The transmissibility of the disease to cattle by blood inoculation was confirmed by Rinjard (1935), who was able to infect five out of eight cattle by the inoculation of large quantities of virulent blood (200 to 500 c.c.). On the other hand, Dobberstein and Hemmert-Halswick (1928), and Stolnikof (1933) failed to infect cattle by blood inoculation.

Zanucchi (1934) recorded successful transmission to lambs and goats by subcutaneous inoculation of blood or gland suspension and contact infections in two lambs, one goat and one calf. From East Africa, Lichtenheld (1910) described a " malignant-catarrh-like " disease, which was said to have made its first appearance in 1905. He stated that it differed from the European form in that it occurred as an epizootic with a high mortality (75 per cent.). A further differentiating feature was the prominence of hæmorrhagic intestinal lesions, as opposed to lesions of the upper respiratory tract. At this time, it is to be noted, a good deal of confusion existed as to the occurrence of rinderpest in German East Africa.

In Kenya and Tanganyika the existence of " snotsiekte " has been recognised by veterinary officers working in the Masai Reserve for a number of years. Mettam (1923) in his report attributes the occurrence of " snotsiekte " in cattle to contact with blue wildebeest, which though they do not themselves show symptoms of the disease appear to act as carriers of the virus. On one occasion he was able to transmit the disease with the blood of an apparently healthy wildebeest that had been shot in the Orange Free State. In the Masai Reserve of Kenya and Tanganyika cases of " snotsiekte " in cattle occur annually in the months of April, May, June and July, which is the period during which the wildebeest calve.

Curiously enough, the Masai profess to recognise the connection between wildebeest and " snotsiekte," and use the same Masai word for wildebeest and the disease of cattle. One of their beliefs is that " snotsiekte " is contracted by cattle only when they graze over ground contaminated by the after-birth of the calving wildebeest. Another version is that cattle contract the disease when they graze over ground upon which the wildebeest calf has lain and shed its coat. The second version fits remarkably well with Mettam's idea that wildebeest lice may act as vectors of the causal agent.

At this laboratory we have from time to time seen cases of a relatively mild disease which we have diagnosed as malignant catarrh. The affected animals have come from farms so situated as to preclude any possibility of contact between cattle and wildebeest. On these farms cases of the disease have been confined to calves and young weaned animals. In an outbreak of this type, investigated in 1933, the disease appeared to be transmissible by blood inoculation (Daubney, 1934) but the incubation period was extremely variable and might be as long as 13 weeks. The average incubation period between inoculation and temperature reaction was between nine and ten weeks. No fatal cases were produced experimentally, and the infection died out at the third subinoculation. Since this appears to be the only record of transmission of Goetze's mild form of the disease, we have summarised the results of attempts to transmit with this and similar mild strains for the present note.

The irregularity with which positive results have been obtained helps considerably towards an understanding of the conflicting views on transmissibility held by different European workers.

" Mild " Strains of Malignant Catarrh.

Nakuru Strain.

In January 1933 the owner of a farm in the Nakuru district reported that he had lost between 30 and 40 calves and weaners during the course of the year. Clinically the disease was con-

sidered to resemble rinderpest in some respects. Eventually three animals were sent to the laboratory for observation. They were grade calves between three and six months old and all appeared to be suffering from the same disease. There was a catarrhal discharge from the eyes and nose, and in the mouth diphtheritic deposits almost covered the tongue and gums. All three animals showed patches of excoriation on the skin of the neck, inside the axillæ and beneath the tail; at these sites the skin was thickened and there was exfoliation of the epithelium. Since they all appeared likely to recover, one of them, No. X8345, was killed for *post-mortem* examination. In addition to the lesions observed during life, this animal showed faint hyperæmia and elongated erosions of the mucous membrane of the fourth stomach, hæmorrhagic spots in the cortex of the kidney, great enlargement of the mesenteric glands, some of which showed hæmorrhage into the cortex, congestion of the nasal mucosa and necrosis of the mucous membrane over the turbinate bones, and congestion of the meninges of the brain. Histologically some of the blood vessels of the kidney showed a leucostasis, mainly of lymphocytes.

Blood from this animal and the other two, Nos. X8344 and X8346, was pooled and inoculated intravenously into six cattle, Nos. X6025, X6881, X6823, X6574, X7560 and X7365, on January 7th, 1933. Four of the inoculated animals reacted in from six-and-a-half to ten weeks, one gave a doubtful reaction nine weeks after inoculation and the remaining one gave no reaction. The reactions were marked by a fairly high temperature, lasting from three to eight days; nasal and lachrymal discharges, at first watery and later, in some cases, muco-purulent; a blistered appearance of the muffle; hyperæmia of the mouth with ulceration of the gums and commissures of the swollen lips; enlargement of the superficial lymphatic glands and enlargement and hardening of the hæmolymph glands. Clinically the condition was never a serious one, and all the animals recovered. In one of them, however, there was a relapse to the infection three-and-a-half weeks later.

Blood drawn from X6881 reacting six-and-a-half weeks after inoculation, temperature 104·2°, was inoculated in 5-20 c.c. doses on 23.2.1933 to six cattle, Nos. X8082, X7927, X7928, X8335, X8363 and X8508. Two of them, X8082 and X8335, reacted to the inoculation at six and 13 weeks respectively. X7928 gave a doubtful reaction during the eighth week, and the remaining animals did not react. X8335, which reacted 13 weeks after inoculation, exhibited typical symptoms and was killed ten days after the temperature fell. For some days prior to his destruction he had shown a copious thick nasal discharge and marked swelling of the sub-auricular lymphatic glands. The muffle was blistered and there was muco-purulent discharge at the inner canthus of the eyes. On *post-mortem* examination the mucous membranes of

the nose and sinus were found to be greatly congested. All the lymphatic glands were enlarged, hæmorrhagic and surrounded by a gelatinous œdema. The meningeal vessels were intensely injected and there was a marked œdema of the meninges. Histologically the brain showed a perivascular lymphocytic infiltration.

On 25.5.33 blood drawn from X8335 was inoculated in 20 c.c. doses to seven cattle. This blood failed to provoke any reaction, although the inoculated animals were kept under observation for a period of four months, and the strain was lost.

In addition to the blood inoculations from X8344, X8345 and X8346, brain tissue from X8345 was inoculated into the anterior chamber of the eye of five cattle and subdurally into twelve rabbits. No reactions resulted although both lots of experimental animals were kept under observation for a period of four months with weekly weighings.

Fig. 1.—Grade bull No. 588. Age 2 years. Inoculated into the left prescapular gland on 29.8.35 with gland tissue from calf 841. Reacted 26.9.35. Died 3.10.35. Photographed 28.9.35. Passage II.

Masai Strains.

On 12.4.33, in response to a request for material from cases of snotsiekte, a sample of citrated blood was forwarded to the laboratory by the Veterinary Officer in charge of the Masai Reserve. This blood, which was stated to have been taken from a typical case of the disease, was inoculated to eight cattle, Nos. X7535, X7270, X7737, X7868, X7869, X7863, X7581 and

X7017, all immune to rinderpest. Seven of these animals reacted on the seventh and eighth days. Four of them showed temperature reactions lasting from three to six days, and all showed the characteristic symptoms: nasal and lachrymal discharge: blistering of the muffle; hyperæmia and ulceration of the mouth, and swelling of the superficial lymphatic glands and superficial hæmolymph glands. An attempt to carry the virus through another passage by subinoculation of blood from X7868, X7017 and X7737 to four cattle failed.

On 3.6.35 a second sample of blood from a case of snotsiekte in the Masai Reserve was forwarded to the laboratory. Large doses of this blood were inoculated to three cattle but no reactions were produced.

Second Nakuru Strain.

On 12.7.33 a calf was received from the owner of a farm that marches with the boundary of the one from which the first Nakuru strain had been obtained. Blood from this animal, No. X8852, which showed symptoms typical of malignant catarrh, was inoculated to three cattle, Nos. X8830, X8831 and X8833, on the day of the calf's arrival. All gave doubtful reactions, X8831 and X8833 during the third week after inoculation and X8830 during the tenth week. An attempt to subinoculate this strain from animals X8831 and X8833 during the doubtful reactions failed.

Kabete Strain.

Two further cases occurred on one of the laboratory farms in November, 1933, among a herd of 75 heifers that had been purchased some seven months previously in the Kavirondo Reserve. Both these heifers died, X8330 on 17.10.33 and X8293 on 25.10.33. Both were emaciated, and X8293 showed the typical skin lesions of the "mild" form. The lesions found *post-mortem* were stomatitis, pharyngitis, rhinitis, injection of the mucosal vessels of the larynx, with ulceration in X8330; prescapular lymphatic glands greatly swollen and hæmorrhagic, hæmolymph glands enlarged and firm. In X8293 the liver was slightly swollen and mottled in appearance, the kidneys were also mottled and contained small "infarcts," and the fourth stomach was congested and studded with pin-point hæmorrhages. From heifer X8330 two animals, Nos. X8619 and X8759, were inoculated subcutaneously with gland tissue on 17.10.33; two others, Nos. X8621 and X8756, were similarly inoculated with spleen pulp. Three of the inoculated animals did not react; but X8756 gave a doubtful reaction, which was complicated by the occurrence of redwater, during the second week. An attempt to subinoculate this strain to four other cattle failed. From the second heifer, No. X8293, a mixture of blood and lymphatic gland pulp was inoculated

subcutaneously to two cattle, X8921 and X8925. No reactions were produced.

As a result of these preliminary observations, it was concluded that we were dealing with a specific disease answering to the description given by Goetze for the " mild " form of malignant catarrh, that the infection was with difficulty transmissible to cattle by blood inoculation after an extremely variable but usually lengthy incubation period, and that it showed a marked tendency to die out in the course of successive subinoculations. With the exception of two strains obtained from the Masai Reserve, all the material had come from farms where contact between cattle and wildebeest was impossible.

Fig. 2.—Grade steer No. 497. Age 10 months. Inoculated with pooled brain and gland tissue from bovine 839 into left prescapular gland on 13.10.35. Reacted 8.11.35. Died 21.11.35. Photographed 14.11.35. Passage IV.

New Strain of the " Head-and-Eye " Type.

During 1935 we have been fortunate in obtaining an extremely virulent and readily transmissible strain, which reproduces the disease in what Goetze has appropriately termed the " head-and-eye " form. The actual origin of this strain is obscure.

On 30.5.35 an adult ox was brought to the laboratory for diagnosis from a farm in the Mua Hills area, about 35 miles from Nairobi. Clinically the ox was suffering from *muthioko* or turning sickness, and a tentative diagnosis to this effect was confirmed by histological examination of the brain when the animal was destroyed five days later. At the same time it was noticed that the

superficial lymphatic glands were greatly enlarged and there was a heavy muco-purulent nasal discharge. The cornea of both eyes was opaque, but this did not attract much attention since corneal opacity is a frequent lesion in uncomplicated cases of turning sickness. Inoculation of brain from this ox, made in an effort to transmit turning sickness, resulted in the death of one out of the three cattle inoculated into the prescapular gland. This animal, X7598, died 28 days after inoculation and *post-mortem* was found to be suffering from meningitis. The brain contained bi-polar organisms. The prescapular lymphatic glands were greatly enlarged and showed the crimson hæmorrhagic areas that we have come to regard as typical of malignant catarrh; but unfortunately their significance was not at the time appreciated.

On 14.5.35, a young wildebeest calf that had been deserted by its dam on the Nairobi aerodrome was brought to the laboratory. This calf died 13 days later from diarrhœa, due in all probability to the unsuitability of the method of rearing. During the time that it was at Kabete the wildebeest calf had been in contact with a number of young calves all of which were kept in a loose box separated by a rough corrugated iron partition from the box in which the ox suffering from turning sickness had lived for five days. Sixty-three days after the death of the wildebeest and 55 days after the death of the ox, one of the calves, No. 496, was reported sick and was found to have enlarged superficial lymphatic glands; smears were examined for east coast fever, but no parasites could be found. Three days later, on August 1st, the calf died. On the day of its death it had a catarrhal discharge from the eyes and nose. On *post-mortem* examination a hæmorrhagic patch was found on the lower gum, and fine hæmorrhages and hæmorrhagic ulcers were present in the abomasum. The liver was swollen and the vessels of the wall of the gall bladder were injected. The lungs showed œdema, hyperæmia and scattered pin-head hæmorrhages. The epicardium was petechiated. All the lymphatic glands were swollen and more or less hæmorrhagic, the prescapular containing large crimson areas. There was an increase in cerebro-spinal fluid and petechiation of the meninges, and the external surface of the rumen showed a rough hæmorrhagic patch on the dorsal aspect of the left sac.

It is from this calf that our virulent strain has been derived. Unfortunately its true origin must remain unknown.

Transmission.

Starting with the spontaneous case of "snotsiekte" or malignant catarrh in calf No. 496, we have been able to study the course of the disease in 33 artificially infected cattle, in all of which the infection terminated fatally. Infection has been successfully transmitted by subcutaneous inoculation of large quantities of defibrinated blood, 110-275 c.c., taken during the febrile

reaction; by intraglandular inoculation of suspensions of brain or lymphatic gland pulp collected at death; and in a single instance by simple contact. In the course of four serial passages, 18 cattle have been infected by blood inoculation, five by brain inoculation, six by gland inoculation and four by mixed brain and gland inoculation. Of 36 animals inoculated with fresh material, 33 reacted and died, one died from an intercurrent disease, and two did not react.

Incubation Period.

The average incubation period in 33 cattle experimentally infected has been 20·9 days, with a maximum of 60 days and a minimum of 14. In 23 animals the period was between 16 and 24 days inclusive, in one 14 days, in two 15 days, in six over 25 days and in one 60 days. The period is calculated from the time of inoculation to the first rise in temperature; not infrequently mild catarrhal symptoms appeared three to four days before the beginning of the febrile reaction.

Duration of Illness.

The average duration of the disease from the initial rise in temperature to death or the time of slaughter *in extremis* has been seven to eight days, with a minimum of four days and a maximum of 25. Out of 33 animals, 28 died in less than ten days.

Symptoms.

The symptoms in experimentally produced cases do not differ from those described in natural cases. A typical temperature chart is reproduced. Three or four days before the temperature rises the superficial lymphatic glands may feel more tense than normal and it is usual at this stage to find slight watery discharge from the eyes and nose. With the initial rise in temperature the sclerotic and buccal mucous membranes may be hyperæmic, the nasal and lachrymal discharges increased, and a certain degree of photophobia may be present. The discharges may now be so copious as to drip continuously on to the ground. As the disease progresses photophobia becomes intense and the discharges thicken, forming blobs or long strings of yellow muco-pus. In most cases there is dyspnœa with stertorous nasal and tracheal râles. In reacting animals that are kept out of doors the symptoms of dyspnœa are aggravated by any slight exertion, and flecks of blood are frequently seen in the nasal discharge.

In about a third of the animals the early hyperæmia of the mouth recedes without any eruption. In the remainder it gradually increases in intensity and leads within two or three days to a more or less generalised ulcerative stomatitis, which may cause excessive frothy salivation and champing movements of the jaws. The muffle is usually dry and hyperæmic, and in some animals superficially ulcerated.

A thick muco-purulent nasal discharge is a more constant feature than a thick discharge from the eyes. When there is marked photophobia the lachrymal discharge is usually watery and copious; but in a few animals one finds only a large bead of dried discharge at the inner canthus. The vessels of the sclera are generally markedly injected; more rarely there are pin-head petechiæ on the conjunctiva and the edge of the sclera. Quite soon after the appearance of lachrymal discharge there may be a dense opacity of the aqueous humor, or the fluid of the anterior chamber may contain floccules, the cornea remaining unaffected. In other animals the fluid in the anterior chamber may remain clear, but an acute keratitis is frequently observed and occasionally one sees ulceration.

In a percentage of cases the whole head swells noticeably and the swelling persists until death. In other animals there may be a diffuse œdematous swelling of the intermaxillary space which may extend down the ventral surface of the neck.

Diarrhœa has been observed twice only in 33 cases of the disease; most of the animals are rather constipated.

In the great majority of cases the superficial lymphatic glands are considerably enlarged. Frequently they can be seen. On palpation they are firm and may present irregular protuberances, especially on their proximal surfaces. In some animals, more particularly in the less acute cases, the hæmolymph glands of the prescapular and mid-cervical groups may be easily palpated.

Symptoms referable to lesions of the central nervous system are frequently noticed soon after the first rise of temperature. Those most often observed are muscular stiffness, tremors, weaving of the head and arching of the back. In advanced cases the gait is very unsteady and the animal may cross its hind legs and stumble when walking. Marked drowsiness is common, the animal standing with its head held low as if sleeping. When startled by a sudden noise hyperæsthesia is shown. Sensitivity is unimpaired throughout.

Although feeding may continue until a day or so before death, animals lose condition rapidly after the commencement of the febrile reaction. Skin lesions have not been observed with this strain; in fact it is unusual to find the coat rough.

The time of appearance of symptoms in relation to the temperature reaction affords help in the clinical differentiation of this condition from rinderpest. Whereas in malignant catarrh pronounced symptoms may be present on the day that the temperature rises, in rinderpest muco-purulent lachrymation, nasal discharge and hyperæmia of the gums do not, as a rule, appear until after the temperature begins to fall; and ulcers do not appear on the gums until the fever has completely subsided. Another important difference between the two diseases is the rarity of diarrhœa in malignant catarrh.

Post-mortem *Lesions*.

Digestive System.—In about one-third of the animals that have come to *post-mortem* examination the mouth has been normal. Of the remainder approximately one-half show submucous hæmorrhages on the tongue, diffuse ulceration of the gums, tongue, cheeks and palate, with extensive cheesy diphtheritic deposits. In the remaining animals the mouth lesions are milder in character; they may consist of hæmorrhagic patches on the lower gum or on the tongue, or there may be ulceration confined to the lower gum or to the tongue and roof of the mouth, or there may be merely a prominent line of hæmorrhagic congestion at the base of the incisor teeth.

In approximately the same proportion of cases the pharynx is normal. In the remainder the changes may vary from the presence of a few petechiæ on the tonsils to extensive ulceration, erosion and diphtheresis of the whole of the pharynx. In the abdominal cavity the surface of the gastric omentum shows in the majority of cases a roughened patch with diffuse sub-serous hæmorrhage, varying in size, but frequently as large as the palm of the hand. A similar patch is often found on the wall of the left sac of the rumen. Occasionally pin-head petechiæ are scattered through the omentum and mesentery.

In only a very few animals is the mucous membrane of the abomasum unchanged. The changes found vary from hyperæmia with a few scattered petechiæ to a complete peppering of the whole of the mucous membrane with hæmorrhages, pin-point and lenticular. On the whole the lesions in the fourth stomach show a remarkable similarity to those seen in sheep dead of Nairobi sheep disease.

The small intestine does not as a rule show any severe lesion. In a few animals the mucosa is catarrhal and studded with petechiæ; but in many cases the lesions are confined to small hæmorrhagic patches. Again, in the majority, the mucous membrane of the cæcum and rectum is unchanged; in a few animals petechiæ are found in the cæcum, and in others small areas of congestion. When present, lesions in the rectum are confined to diffuse hyperæmia or a few pin-head hæmorrhages on the rugæ. Examination of the cæcum and rectum is of considerable help in differentiating this disease and rinderpest.

There is almost constantly a marked liver lesion which is manifest to the naked eye in swelling of the organ and a more or less distinct mottling, usually visible through the capsule and at times obvious on examination of the cut surface. The mottling consists of small whitish spots which on histological examination prove to be aggregations of infiltrating cells surrounding the blood vessels of the organ.

In most animals the wall of the gall-bladder is unchanged; but in a few cases changes, varying in intensity from injection of

the mucosal vessels to extensive petechiation, are seen. The bile on the other hand is rarely normal, and is usually dark green, thick and grumous.

Urinary System.—The kidneys always present a characteristic lesion. In about half the animals there is mottling, similar to that occurring in the liver: and in the remainder, in addition to the mottling, there may be larger white or yellowish "infarcts" which resemble those of east coast fever but are usually larger and more irregular in outline. Petechiæ are only occasionally seen and the organs are not as a rule noticeably congested.

In about a third of the animals the bladder is normal. In the remainder the changes vary in intensity from patchy hyperæmia with capillary arborescence to an intense hæmorrhagic inflammation, which may be accompanied by innumerable pin-point- to pin-head-sized petechiæ or by extensive submucous extravasation.

In nearly every case the urine, which is acid, contains albumen, varying from 0·5 to 3·0 parts per thousand (Esbach); an occasional sample may contain red cells or hæmoglobin.

Respiratory System.—In only one animal has the nasal mucous membrane been healthy. Usually the changes in the nose are most marked on the turbinated bones and septum. They vary from congestion to extensive ulceration, occasionally with numerous petechiæ or heavy diphtheritic deposits. In some animals the frontal sinuses and horn cores are strongly congested and catarrhal.

In about 60 per cent. of cases the larynx shows intense congestion and petechial hæmorrhages. In other cases there may be a diffuse hyperæmia with a croupous membrane or a plug of muco-pus. Ulceration is occasionally observed.

The trachea is frequently congested and there may be hæmorrhages along the dorsal line.

In the thoracic cavity petechiæ are occasionally seen beneath the parietal pleura.

In general the lungs are œdematous, and in about half the animals there are small areas of atelectasis, hepatisation, or even purulent broncho-pneumonia. The lung lesions are always confined to the anterior parts of the organ, *i.e.*, the cephalic, cardiac, and intermediate lobes, and the antero-ventral angle of the main lobes.

Circulatory Systems.—Petechiæ are present on the epicardium in about half of the cases. They are particularly frequent in the region of the coronary grooves. On the endocardium they are more rarely seen, and when present are always in the left ventricle. Occasionally the myocardium shows pale areas of degeneration and infiltration.

The blood appears normal and coagulates within the usual time. The spleen is usually somewhat swollen, and there are frequently subcapsular hæmorrhages, most marked near the free

border. In a small proportion of cases there is true *tumor*. On section of the organ the Malpighian bodies generally appear larger than normal and project from the cut surface. In a few examples the pulp has been "jammy," and in two old-standing cases there has been a change closely resembling amyloid degeneration.

Throughout the body the lymphatic glands are everywhere enlarged. On section they all appear moist, and in about three-quarters of the animals they contain patches of congestion or multiple small hæmorrhages. The patches of congestion are bright crimson in colour and may involve one or more follicles, frequently reaching a diameter of 1½ inches in the prescapular glands. As a general rule there is a greater tendency towards hæmorrhage and congestion in the glands of the throat than in those of other parts, and in a few animals the change is restricted to the retropharyngeal glands. In these glands the congested area is greater, and it is not uncommon to find the whole gland more or less evenly affected. Although they are not invariably present, the round crimson areas in the prescapular and precrural glands and the general hæmorrhagic condition of the throat glands have considerable pathognomonic significance. The development of protuberances on the proximal surface of the prescapular glands has already been mentioned. These protuberances consist of groups of follicles in the cortical region which have become enlarged to a greater degree than the remainder; the follicles in question may be completely hæmorrhagic and dark crimson in colour.

When an animal has been inoculated intraglandularly the inoculated gland is invariably found to be enormously enlarged and hæmorrhagic.

The glands of the thoracic and abdominal cavities although constantly enlarged do not commonly show congestion or hæmorrhage.

With the general swelling of the lymphatic glands, unnamed lymphnodes which normally would be too small to be recognisable with the naked eye may reach the size of a walnut. Further, and more particularly in less acute cases, all the hæmolymph glands may be considerably enlarged, *e.g.*, a chain of hæmolymph glands varying in size from that of a pea to a hazel-nut may be found running superficially upwards from the prescapular gland parallel to the anterior border of the scapula.

The bone marrow has been examined in only a few cases, but has always appeared unchanged.

Reproductive Organs.—Most of the animals employed in the experiments have been steers, but in a few instances bulls or heifers have been inoculated. Petechiæ have been present in the ovary in one case, and in one bull petechiæ were seen in the substance of the testicle. No definite lesions were observed in the vagina.

Nervous System.—On severing the head from the neck at the occipito-atlantal joint, the cerebro-spinal fluid usually appears to be under a greater pressure than normal. In the cranial cavity there is an excess of fluid under the dura mater and the meninges are moist and frothy. Frequently there is a pronounced smell resembling that of meat broth.

In about a fifth of the animals examined the surface of the brain itself has appeared anæmic; in the remainder there has always been some degree of congestion or gross hæmorrhage. Occasionally large extravasations of blood may be present in the form of a cap, covering the posterior third of both cerebral hemispheres and the cerebellum. The clot may be sub-arachnoid, or situated between the *dura* and the surface of the cranial vault, or in both together. Similar gross hæmorrhages have been described by Dobberstein (1925), who concluded that they were due to trauma produced by stunning. None of our animals has been killed by stunning, and these plate-like extravasations have been seen in animals that have died. They have been present in roughly one-fifth of the animals examined. Smaller coagula are frequently found surrounding the choroid plexus between the posterior surface of the cerebellum and the medulla, and between the occipital poles of the cerebral hemispheres and the cerebellum. In one instance clots occurred in the lateral and third ventricles.

Rather more commonly one finds the superficial vessels strongly injected and the surface of the organ studded with petechiæ, which may be up to 2 mm. in diameter. Occasionally, in a brain that is not congested, one notices more or less bilaterally symmetrical yellowish-orange areas, which are frequently 3 to 5 cm. in diameter and slightly below the general surface level. They are usually situated in the region of the lateral fissure.

On section of a congested brain there may be indications of minute hæmorrhages into both grey and white matter.

In several cases there has been a diffuse bluish sub-arachnoid hæmorrhagic effusion extending from the medulla almost to the cervical enlargement of the cord; and in one animal a similar effusion was found in the region of the *cauda equina*. A similar lesion is also not uncommon beneath the sheaths of the brachial and sciatic nerve trunks.

Musculature.—The carcase is always emaciated. In cases that have shown swelling of the head or œdema of the submaxillary space one finds an amber-coloured gelatinous exudate under the skin and infiltrating the adjacent fascia and muscles. Frequently, in the absence of detectable swelling, a similar exudate surrounds the prescapular lymphatic glands. This subcutaneous œdema is reminiscent of the classical œdema of hæmorrhagic septicæmia, at least in so far as the more acute cases of that disease are concerned.

Occasionally the whole carcase is markedly icteric, while in other animals the muscles may have a parboiled appearance. None of our cases has been complicated by anaplasmosis or red-water, although many of the animals employed have undoubtedly been carriers of both parasites.

Histo-pathology.—A considerable amount of histological material from natural and experimental cases of the disease has already been accumulated, but it is not our intention at this stage to enter upon any detailed description of the histology of the lesions. We shall confine ourselves to a brief ennumeration of the gross changes observed in the different organs.

It is to be noted at the outset that in animals that have died from the so-called "mild" form of the disease, it may be impossible to detect any of the changes that are now described as typical of the more virulent "head-and-eye" form. The most striking change in all the organs is perivascular infiltration, largely lymphocytic, and affecting indiscriminately arteries and veins of all sizes.

The infiltrative lesion is most constantly found in the kidney, where there is always marked perivascular infiltration surrounding some of the medium-sized vessels in the boundary zone. The infiltrating cells are free in the interstitial tissue surrounding the vessels; polymorph cells are extremely rare in the cuff. In addition to infiltration that is definitely perivascular in distribution there are usually irregularly-shaped areas of interstitial infiltration in the cortex, the location of which cannot be definitely assigned to the presence of vessels larger than capillaries. These areas of infiltration may be so dense and at the same time so large as to appear macroscopically as white "infarcts." The glomeruli in an affected kidney show an increase in cellularity, and there may be pressure necrosis of the epithelium in tubules enclosed within, or adjacent to, strongly infiltrated areas. In older standing cases groups of two or three tubules may coalesce and form small cysts that are devoid of any epithelial lining.

Next in order of frequency liver lesions are found; again the main lesion is perivascular infiltration, which may be massive. The infiltrating cells surround artery, vein and bile duct in the portal canal; they may be wholly confined within the greatly distended Glisson's capsule or at points they may escape into the lobule proper. The central veins are less commonly affected. At times the liver cords are considerably more widely separated than normal and the sinusoids may contain an excess of round cells, but they are never plugged; the Kupfer cells appear in these cases to be swollen and active. Parenchymatous changes in the liver are either extremely slight or absent.

Another almost constant feature is cuffing of vessels in both grey and white matter of the brain and cord. The cuffing cells may be confined to the Virchow-Robin space, and may be seen

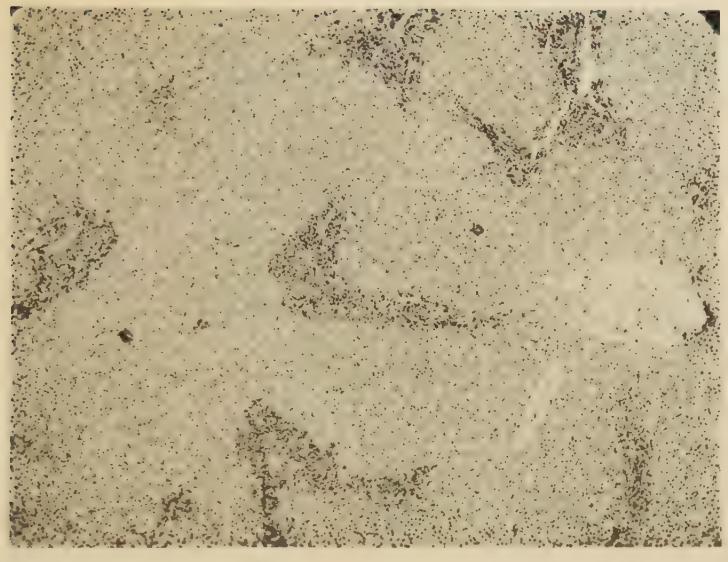


Fig. 3.—Bovine 839. Passage III. Liver ×60. Methylene blue and orcein.

Fig. 4.—Rabbit ND3. Passage III (subdural). Cord ×60. Hæmatoxylin and oesin.

migrating through the vessel wall, often in considerable numbers; less frequently they escape into the brain tissue, forming a wide cellular zone around the vessel. In some instances we have found what appear to be purely focal infiltrations, generally in the grey matter, and occasionally satellitism of the neurons has been in evidence. Similar lesions are found in the cord at all levels. In the brain, infiltration may be particularly marked round the meningeal blood vessels in the sulci. Joest-Degen corpuscles have never been found, but we have not yet had the opportunity to make a minute study of the neurons for cytological changes.

Sections of the lymphatic glands show congestion, œdema, and frank perivascular infiltration of vessels in the trabeculæ. There is marked activity in many of the germinal centres with the production of lymphocytes, chiefly of the small variety.

Similar perivascular infiltrations may be found in the interstitial tissues of salivary glands, in all parts of the adrenals, and around the smaller arteries and veins of the striped and unstriped muscles.

Occasionally in the brains of animals that have survived rather a long time after the onset of symptoms, bacteria are found, but in the great majority of cases the brain collected at death has failed to give cultures on all the usual media, including chocolate agar. This has been true of every brain from which a rabbit strain has subsequently been established (*vide infra*).

Contact Infection in Cattle.

Neither Goetze and Liess (1929) working with a transmissible European strain, nor Mettam (1923) in his experiments with snotsiekte, could demonstrate the infection of cattle by direct contact. Both were driven to suggest that the virus might be conveyed from animal to animal by some arthropod vector. Each in turn tried to confirm this hypothesis, Mettam employing cattle lice and lice from the blue wildebeest, Goetze and Liess (1929) cattle lice, and Goetze (1930) sheep keds; in no case with any success.

To account for the spread of the disease in the absence of obvious direct transmission from infected to healthy cattle, Goetze and Liess (1930) and Goetze (1930) adduced what on the face of it seems to be conclusive evidence that apparently healthy sheep can, by close contact, transmit infection to cattle without themselves showing symptoms of infection. Similar views concerning the possible part played by wildebeest in South and East Africa have been mentioned earlier in this paper.

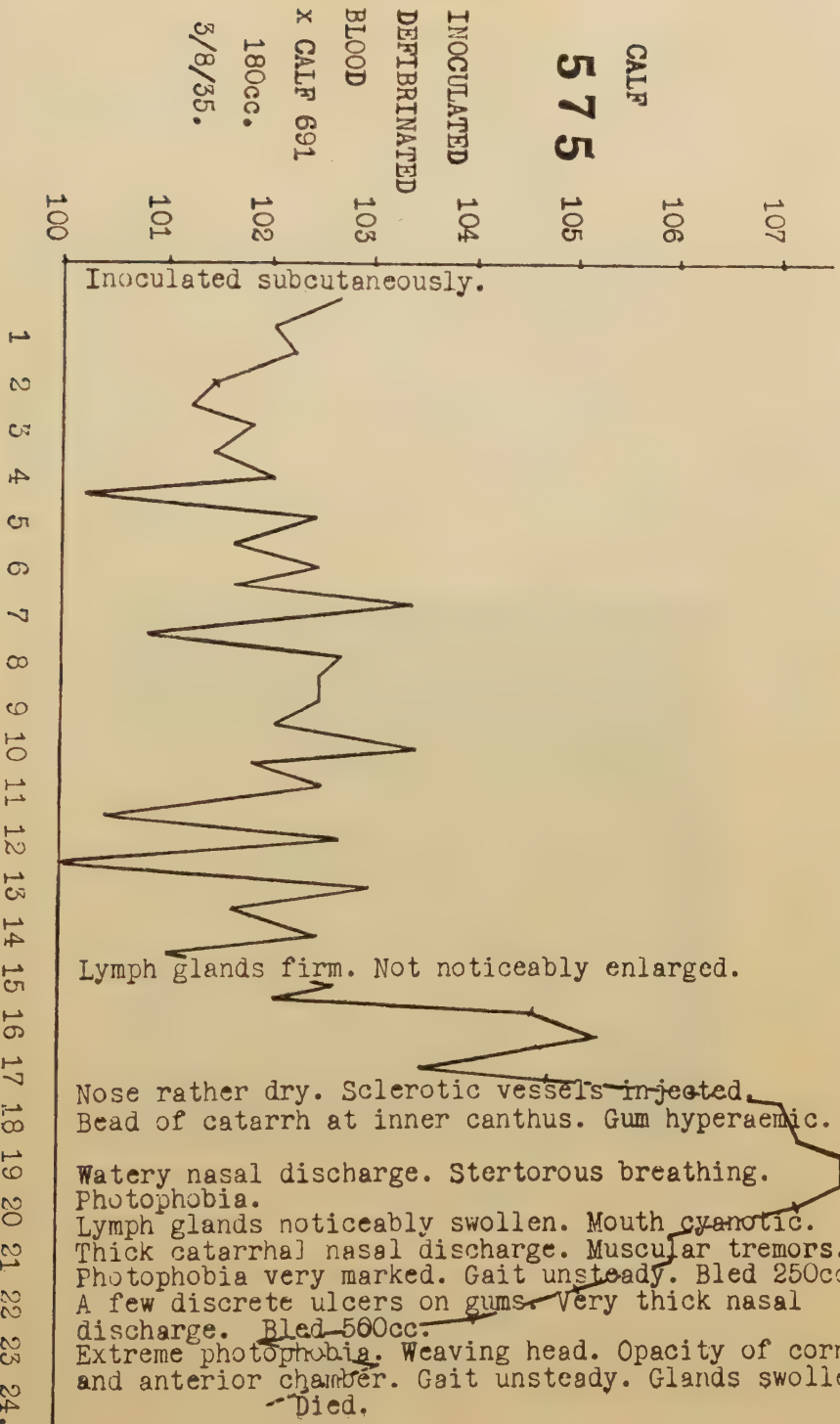
Zanzucchi (1934) on the other hand has reported successful transmission of the disease by blood inoculation to sheep and goats; and subsequently he obtained infection in a calf as a result of contact with goats, which had themselves been infected by contact with blood-inoculated lambs.

In the course of experiments with the virulent strain of the " head-and-eye " form, we have observed an undoubted case of contact infection. It might be added that the cattle at the laboratory are dipped every three days when not on experiments indoors; ticks are very rarely found, and lice are never seen on the laboratory stock. It seems unlikely, therefore, that in this instance transference of the disease could be effected by any of the common ecto-parasites.

Two calves, Nos. 783 and 1084, were exposed to contact with calves Nos. 575 and 757 on 24.9.35; three days later, on the death of No. 575, they were moved to the box occupied by Nos. 66 and X8556, and again on the death of these animals they were transferred to another heavily infected box. Contact was very close, the contact animals eating food grossly contaminated by the muco-purulent discharges from sick animals. On 17.10.35, 23 days after the first exposure, calf 783 gave a marked temperature reaction. On the following day he showed mucous nasal discharge, dirty eyes and slight salivation. His mouth was normal. On the next day his head was swollen, and he stood with head extended and back arched. There was a profuse muco-purulent discharge from the eyes and nose, and sclera showed petechiæ and the mouh was hyperæmic.

On 21.10.35 his temperature fell, the eye and nose discharges were less and the animal fed a little. His head was still very swollen and he showed almost continuous muscular tremors. For eleven days afterwards his morning temperature was normal, and it was thought that he would recover. After a week, however, his condition became worse; there was very profuse nasal discharge, very marked salivation and dribbling of urine. Nervous symptoms became more prominent, he tottered when induced to walk, and had obvious difficulty in swallowing. He was destroyed on 2.11.35.

On *post-mortem* examination the alimentary tract and the thoracic organs appeared normal. There was intense congestion of the turbinated bones and nasal septum, particularly marked on the left side. The liver was pale on section, the bile dark green and grumous; the kidneys were infiltrated and showed typical white " infarcts " up to 1 cm. in diameter; the lymphatic glands were normal but the hæmolymph glands were swollen and hard. There was an increase in cerebro-spinal fluid in the cranium and occasional fine hæmorrhages on the surface of the cortex. On the medulla and pons there was a flat sub-arachnoid coagulum; this clot extended forwards under the right hemisphere, and posteriorly surrounded the origin of the right fifth cranial nerve. Another clot started around the roots of the ninth and tenth cranial nerves and extended both dorsally and ventrally to the cervical enlargement of the cord.


CALF
575
INOCULATED
DEFIBRINATED
BLOOD
x CALF 691
180cc.
5/8/35.
100
101
102
103
104
105
106
107
Inoculated subcutaneously.
Lymph glands firm. Not noticeably enlarged.
Nose rather dry. Sclerotic vessels injected.
Bead of catarrh at inner canthus. Gum hyperaemic.
Watery nasal discharge. Stertorous breathing.
Photophobia.
Lymph glands noticeably swollen. Mouth cyanotic.
Thick catarrhal nasal discharge. Muscular tremors.
Photophobia very marked. Gait unsteady. Bled 250cc.
A few discrete ulcers on gums. Very thick nasal
discharge. Bled 500cc.
Extreme photophobia. Weaving head. Opacity of cornea
and anterior chamber. Gait unsteady. Glands swollen.
Died.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24.
Days after Inoculation.

Histological examination of liver, kidney and brain confirmed the diagnosis of malignant catarrh.

The other calf, No. 1084, has shown neither a temperature reaction nor definite symptoms during the two-and-a-half months that he has been exposed to infection.

Infection of Rabbits.

Concerning the transmissibility of the infection to rabbits there has been an even greater divergence of experience than that recorded in experiments on bovines, and the problem has been still further complicated by the claims which have been advanced that malignant catarrh in rabbits is either Borna disease or alternatively a very closely related disease. In part these claims have been based upon the identity or similarity of the symptoms shows by rabbits infected with the two diseases, and in part upon the finding of the so-called Joest-Degen corpuscles in the ganglion cells of Ammon's horn from rabbits that have died after subdural inoculation with brain tissue taken from fatal cases of malignant catarrh in bovines.

Fig. 5.—Rabbit 3PCc. Inoculated subdurally with brain emulsion of rabbit 2PCb on 25.10.35. Died 25.11.35. Photographed 24.11.35. Passage III of a strain recovered by intraperitoneal inoculation of blood from bovine 575 (Passage II Cattle).

Ernst and Hahn (1927) inoculated a rabbit subdurally with brain taken from a case of " Dummkoller " in an 18-month-old bovine that on *post-mortem* examination had shown circumscribed areas of infiltration and hæmorrhages in the brain, but no definite perivascular infiltration. The rabbit became ill with symptoms

characteristic of Borna disease 54 days after inoculation and died seven days later. The disease was transmissible in series in the rabbit, and Joest-Degen corpuscles were demonstrated. They concluded that malignant catarrh of cattle is produced by a virus that is very closely related to, or even identical with, that of Borna disease of horses.

In a later publication (1931) Ernst reviewed their findings in the light of more recent work by Zwick, Seifried and Witte, who had shown that contact infection from rabbits infected with Borna disease to healthy rabbits might occasionally occur. It appears that the original rabbit of Ernst and Hahn's malignant catarrh series had before inoculation been kept in a box with two other rabbits which three weeks previously had been inoculated with the virus of Borna disease but had not then developed symptoms.

The findings of Ernst and Hahn were confirmed and extended by Zwick and Witte (1931), who inoculated rabbits with brain material taken by Goetze from a cow dead of malignant catarrh. Two rabbits were inoculated subdurally, two intramuscularly, and two subcutaneously. One of the two rabbits inoculated intramuscularly became ill with typical symptoms of Borna disease 68 days after inoculation and died eight days later. The brain of this rabbit showed numerous perivascular infiltrations, but no inclusions—these appeared first in the fourth passage rabbits. The virus was carried through eight passages and at the fifth passage two sheep were inoculated intracerebrally; at the sixth passage two horses were inoculated by the same route; at the seventh passage a two-year-old bovine, also intracerebrally; and at the eighth passage a sheep and two goats.

Two of the three inoculated sheep died, 24 and 57 days respectively after inoculation; one horse died 42 days after inoculation, and the other horse, which was immune to Borna disease, survived. The symptoms and *post-mortem* findings in the sheep and the horse are said to have been those of typical Borna disease.

The steer developed symptoms 39 days after inoculation, but had completely recovered seven or eight days later. The symptoms were largely nervous in origin, but Zwick and Witte record serous discharges from the nose and eyes, and excessive salivation. There was no febrile reaction. In the case of the steer they considered that the symptoms, though largely those of Borna disease, included certain features characteristic of malignant catarrh. The fact that the inoculated disease was not typical of malignant catarrh they attributed to the unnatural method of infection. According to Goetze (1934), Stoss (1933) also obtained evidence from experiments with rabbits in support of the hypothesis put forward by Ernst and Hahn.

On the other hand, numerous failures to infect rabbits have been recorded by Dobberstein and Hemmert-Halswick (1928), Goetze and Liess (1934), Stolnikoff (1933), Miessner and Schoop

(1934) and Aleksa (1935). In spite of his having supplied the material for the experiments of Zwick and Witte, Goetze is disposed neither to accept their results as final nor to agree with the hypothesis as to the identity of the two diseases.

In an experiment reported by Goetze (1934) four cattle were "immunised" with infected rabbit brains obtained part from Zwick and part from Ernst. In a subsequent immunity test with virulent blood two contracted infection and died and two completely resisted several inoculations of virulent blood. According to Goetze's experience this was about a normal proportion of takes for unprotected animals.

It will be recalled that earlier in this paper we recorded failure to infect twelve rabbits, inoculated subdurally with brain material from a weakly transmissible strain of the "mild" type.

With the present virulent strain we have succeeded in infecting rabbits, producing characteristic lesions in the liver, kidney, brain and cord. The disease has been transferred from cattle to rabbits seven times out of ten attempts. Of the three failures, in one the inoculated animals have survived, and in the other two they succumbed within a day or two to an accidental bacterial meningitis. Of the seven successful transfers, six were made subdurally with brain tissue as inoculum, and one intraperitoneally with defibrinated blood.

It may be of interest to give details of the first passage rabbit inoculations, in each of the seven transfers in which "takes" were obtained, for comparison with the results of Ernst and Hahn (1927) and Zwick and Witte (1931). In the first experiment two rabbits inoculated subdurally with the brain of calf 496 (spontaneous attack) both died, one on the eighteenth day, and the other on the forty-first day. In the second attempt three rabbits were inoculated subdurally with the brain of calf 575 (second bovine passage); one died on the eleventh day, showing characteristic lesions, and the remaining two survived. From the same calf four rabbits were inoculated intraperitoneally with 4 to 5 c.c. doses of defibrinated blood, drawn on the fifth day of the temperature reaction; one of these died on the eighteenth day, another on the nineteenth day, another on the forty-eighth day, and one survived. This strain is now in its fifth passage. The fourth attempt was made with brain emulsion from calf 757 (second bovine passage) inoculated subdurally into two rabbits, of which one died on the thirty-first day and the other on the thirty-seventh day. This strain is now in its third passage. In the fifth experiment four rabbits were inoculated subdurally with brain from bovine 66 (second bovine passage); one died on the thirty-fourth day, another on the forty-second day, and two survived; this strain has been successfully subinoculated. In the sixth attempt four rabbits were inoculated subdurally, two with brain and two with testicle from bovine 588 (second bovine passage); all four sur-

vived. The rabbits inoculated in the seventh and tenth attempts died of bacterial meningitis. In the eighth attempt four rabbits were inoculated subdurally, two with brain and two with testicle from bovine 1086 (third bovine passage); one of the brain-inoculated rabbits died on the thirty-fifth day and the other on the thirty-sixth day. Both the rabbits inoculated with testicle have survived 45 days. In the remaining attempt four rabbits were inoculated subdurally with the brain of bovine 1085 (third bovine passage); one died on the twenty-seventh day, another on the thirty-fourth day, and two have survived 40 days.

At the time of writing the first recovered strain, *i.e.*, the one recovered from a natural case of the disease, is in its eighth subdural passage. Out of the total of 37 rabbits used in the first six passages of this strain there are only two survivors. Death occurred in from 14 to 50 days, average 20 days. Of the 35 animals that died or were killed *in extremis*, 26 died between the sixteenth and twenty-fourth days after inoculation, five died on the fourteenth or fifteenth days, and four between the twenty-fifth and fiftieth days. Most of the rabbits were considerably over 1,500 gms. in weight at the time of inoculation. The period is appreciably shorter than that recorded by Nicolau and Galloway (1928) for a strain of Borna virus (Zwick)—average period between inoculation and death in rabbits over 1,500 gms. weight 36 days, in rabbits under 1,500 gms. weight 20 days. With Miessner's strain of ovine origin the corresponding figures were 33 and 28 days.

Symptoms of Malignant Catarrh in Rabbits.

In a few of the first passage rabbits symptoms are noticed from one to three weeks before death; but in all later passages it is not usual for an animal to be observed ill for more than three days. Some rabbits are found dead without having appeared sick the previous day; more commonly they are noticed to be ill for one or two days.

When observed, symptoms are usually referable to involvement of the central nervous system; but in occasional animals catarrh of the conjunctival and nasal mucous membranes is marked (*see* Figs. 3 and 4). The first signs of illness are generally sleepiness and disinclination to move. Appetite may be lost early, or in animals that survive for a few days there may be some inclination to feed almost up to the time of death. When an animal in the early stages of illness is persuaded to move its gait may be hesitating or jerky. A little later evidence of paralysis appears, usually in the form of an inability to adduct the limbs. More often the hind-legs only are affected; but not infrequently the hind- and fore-legs on the same side are involved. The animal at this stage can only with difficulty get back on its sternum after being rolled on its side. In an occasional animal normal progres-

sion may be interrupted by a spasmodic kick of the hind-limbs which causes the rabbit to leap high into the air and at times to turn back-somersaults. Other rabbits move in circles, several yards in diameter. Usually the development of complete paralysis of the limbs is not long delayed, and the animal lies on its side in the cage while the paralysed sphincter allows fluid fæces to issue from the anus. Occasionally a sick rabbit will weave its head from side to side, but the characteristic syndrome of meningitis does not occur. We have not seen a rabbit assume the attitude which is said to be typical of Borna disease, that is, to occupy the corner of the cage with its head in the angle between the two walls. Neither have we observed anything resembling the train of encephalitic symptoms described in Borna disease.

Post-mortem Lesions in Rabbits.

In the earlier rabbit passages the *post-mortem* picture in many respects closely resembles that of cattle dead of the disease. When catarrh has been a symptom of the illness there is an intense congestion of the nasal mucous membrane, particularly over the turbinated bones, which may be ulcerated and covered with thick cheesy muco-pus. There is a similar congestion and catarrhal change in the conjunctiva, at times accompanied by keratitis. The meninges are almost always congested and very occasionally petechiated. We have only once observed gross hæmorrhage of the type that is not uncommon in cattle. The liver may appear congested and usually lobulation is very distinct. In the earlier passages the liver may have a mottled appearance, due to peri-vascular infiltrations visible to the naked eye. The kidneys also in the earlier passages frequently show many white infarcts, in addition to a finer greyish-white mottling. Occasionally lenticular hæmorrhages or ulcers are found in the mucosa of the stomach. In a few animals the lungs show congestion and consolidation confined to the anterior lobes. There has been no remarkable enlargement of the lymphatic glands or spleen.

With subdural passage the visceral lesions are progressively reduced.

Histo-pathology in Rabbits.—So far as the changes in the kidney, liver, brain and cord are concerned, the histological changes found are in the earlier passages identical with those seen in cattle. A comparison of the figures on the plate will illustrate this point. In the later subdural passages the lesions of the central nervous system become more severe, and those of the liver and kidney are progressively reduced in severity. Although we have examined brains from a number of rabbits up to the sixth subdural passage, Joest-Degen corpuscles have not yet been found.

In view of the tendency of the virus to become more neurotropic in its affinities when passaged subdurally, we have

attempted to establish a strain by intraperitoneal passage in rabbits. This strain is now in its fifth passage.

It is not proposed in this paper to discuss the nature of the infective agent in malignant catarrh, but we might mention that the brains of rabbits that die of malignant catarrh are usually sterile bacteriologically, and that the infective agent has survived in brain tissue suspended in 50 per cent. glycerine at cold-room temperature for seven days.

Reproduction of the Disease in Cattle.

As Goetze and Liess (1929) justly point out, the final criterion of success in transmission experiments with rabbits is ability to transfer the disease back to cattle. The only previous occasion on which this attempt has been made is that reported by Zwick and Witte (1931), to which reference has already been made.

In an attempt to satisfy ourselves as to the identity of the condition that we were reproducing in rabbits, we inoculated four cattle on 20.9.35 with a suspension of brain from the third rabbit-passage of our first recovered strain. All the cattle were inoculated into the left prescapular gland.

Calf 492, aged nine months, reacted on October 7th, the seventeenth day after inoculation (*see* chart). Except that there was no marked swelling of the superficial lymphatic glands, the symptoms shown by this calf during the six days that it was ill corresponded fully to the picture as observed in animals inoculated directly with bovine material of this strain. There was a copious muco-purulent discharge from the nose and eyes, photophobia, keratitis, swelling of the head, ulcerative stomatitis and unsteady gait. The animal was killed on 13.10.35 as it was feared that it would die during the night.

At *post-mortem* examination the lesions were characteristic: stomatitis, pharyngitis, hyperæmia and petechiæ in the abomasum, patchy hyperæmia of the small and large intestines, pale diffuse patches in the liver, slight enlargement of the lymphatic glands, injection and froth in the meningeal vessels, and hæmorrhages in the grey and white matter of the brain.

Calf 396, aged ten months, reacted on October 5th, the fifteenth day, and died nine days later. The symptoms were again typical of the condition as we have seen it during this investigation. There was a muco-purulent discharge from the nose and eyes, intense photophobia, swelling of the head, ulcerative and diphtheritic generalised stomatitis, stiffness in movement, and in the last two days diarrhœa.

Post-mortem lesions were diphtheritic and ulcerative stomatitis, with erosions on the free portion of the tongue and ulcers resembling those of rinderpest on the upper and lower gums and on the inside of the lips; diffuse hyperæmia and scattered petechiæ in the abomasum, a few old hæmorrhagic striæ in the cæcum and a

CALF
492
INOCULATED
BRAIN EMULSION
RABBIT
ND3 bis a.
20/9/35.
99
100
101
102
103
104
105
106
Inoculated into left prescapular gland.
Off food. Very copious, mucous nasal discharge. Slight lachrymal discharge. Sclera injected.
Intense watery lachrymal discharge. Photophobia marked. Blob of muco-pus at left nostril. Discharges more marked. Both eyes opaque. Left eye almost ruptured.
Profuse muco-purulent discharge from eyes and nose. Gait very unsteady.
Lateral decubitus. Killed in extremis.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23.
Days after Inoculation.

few pin-point hæmorrhages in the rectum. The liver showed numerous fine greyish-white spots, as did also the kidneys. The sinuses and the horn-cores were congested. There was marked congestion of the larynx and trachea, and grey hepatisation of part of the intermediate lobe of the right lung. The epicardium was petechiated. The lymphatic glands generally were not much enlarged, with the exception of the retropharyngeal, which were swollen and hæmorrhagic. The prescapular glands were congested and moist on section and the hæmolymph glands were generally enlarged. The superficial vessels of the brain were congested and the meninges frothy, and there was an increase in the amount of cerebro-spinal fluid.

Calf 421 showed a transient febrile reaction, beginning on the seventeenth day and lasting four days. During this time there were watery discharges from the nose and eyes, and on one day the nasal discharge was muco-purulent. On this day also marked hyperæmia of the mouth was observed. The animal recovered and during the reaction did not lose weight.

The remaining animal, calf 693, had a short febrile reaction beginning on the thirty-fifth day and lasting three days. There were no significant symptoms.

Discussion and Summary.

An account has been given of transmission experiments with strains of malignant catarrh or snotsiekte from Kenya. Two strains at least proved to be transmissible. The first of these transmissible strains, which we could only maintain for two passages in experimental cattle, corresponded clinically and pathologically to Goetze's " mild " form of the disease. It is of interest to note that this strain and four other " mild " strains were obtained from farms where there was no possibility of contact between cattle and wildebeest. It has been shown that the "mild" form as it occurs in this country is irregularly transmissible by blood inoculation, usually after a relatively long incubation period; and that the more virulent " head-and-eye " form is transmissible by blood, brain and gland inoculation, the disease being reproduced with uniform regularity and an exceedingly high mortality. The incubation period in experimentally transmitted cases of the " head-and-eye " form has been similar to that recorded by Mettam for " snotsiekte " in South Africa, and that found by Goetze in Europe for this form of malignant catarrh. The lack of uniformity between different strains, in respect of their transmissibility to cattle, must go far to reconcile the divergencies of opinion on this subject among workers in Europe.

The more intensive study of malignant catarrh which has been made in Europe during the last ten years has resulted in considerable additions to our knowledge of the pathology, ætiology and clinical features of the disease in cattle; and in the light of these

advances the characters that caused Mettam (1923) to describe snotsiekte as an independent disease are no longer valid, and snotsiekte must become a synonym of malignant catarrh.

The experimentally produced disease has not been readily transmissible by contact, but one case of contact infection has been seen in a calf; and it is pointed out that, in view of the short-interval dipping practised at the laboratory, the possibility of ecto-parasites playing any effective rôle in transmission can be discounted.

The disease has been transferred from cattle to rabbits—not once, but seven times out of ten attempts; in six of them by subdural inoculation of brain tissue, and in one by intraperitoneal inoculation of blood. In the early rabbit passages the lesions in the kidney, liver and brain are identical, both macroscopically and microscopically, with those seen in cattle. In later passages the infective agent appears to become increasingly neurotropic, and there is a corresponding reduction in the severity of the visceral lesions. In an effort to retain the viscerotropic characteristics of the original virus, we have attempted to establish an intraperitoneal strain in rabbits. This strain is now in its fifth passage.

For the first time the disease has been satisfactorily reproduced in cattle in typical fatal form by the inoculation of material derived from rabbit passages. Two cattle died out of four inoculated with brain from rabbits of the third subdural passage. None of the inoculated rabbits has shown the symptoms regarded as characteristic of Borna infection in this species, nor have the corpuscles of Joest-Degen been found in the central nervous system.

With regard to the suggestion of German workers that the disease is immunologically related to, or even identical with, Borna disease, we are at the moment arranging to carry out direct immunity tests with the two viruses, but in the meantime we are disposed to agree with Goetze that it is unlikely that the two diseases are identical. The most striking difference between our findings in rabbits and those of Ernst and Hahn and Zwick and Witte is in the production in the earlier passages of our strains of visceral lesions readily recognisable with the naked eye. It is unlikely that such lesions could have been overlooked by experienced workers had they occurred in the German experiments. Our strain, as already stated, reproduces typical malignant catarrh in cattle, whereas in the animal inoculated by Zwick and Witte with brain from the sixth subdural passage of their rabbit strain, the clinical condition bore a greater resemblance to Borna disease than to malignant catarrh. It is possible, however, that the atypical syndrome described by these authors might in part be due to the fact that the virus had undergone a greater number of passages in rabbits before it was returned to cattle, and in part to the employment of the intracerebral method of inoculation.

Our experiments with sheep, guinea-pigs and mice are not yet sufficiently advanced to be included in this note, but we are inclined to believe that we have succeeded in transferring the disease to sheep in a mild form, and we have certainly succeeded in transmitting the "head-and-eye" strain to the other two species.

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FURTHER STUDIES ON THE EFFICACY OF FOWL-POX VACCINES.

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In a previous communication[1] we reported laboratory and field experiments on the relative efficacy of pigeon-pox and fowl-pox vaccine in conferring immunity against fowl-pox infection. As a result of these experiments we concluded that: (1) pigeon-pox vaccine was innocuous, whereas attenuated fowl-pox vaccine involved the risk of secondary lesions; and (2) the latter vaccine produced a more solid and lasting immunity than the former.

During the past two years pigeon-pox vaccine prepared according to Doyle[2] was used extensively in Palestine. It was the practice of poultrymen to vaccinate birds at an early age, usually during the month of March-April. The vaccine was expected to confer immunity against natural infections for a period of at least one year. It was noted, however, that many outbreaks of fowl-pox occurred among vaccinated birds during the months of September, October, November and December. Although the disease in vaccinated flocks assumed a milder course it caused much distress to the poultrymen. In view of the fact that the outbreaks of fowl-pox occurred during the months of September-December, it was assumed that this was the natural season of the infection, and the conclusion from the field observation was that pigeon-pox vaccine does not give a complete immunity against natural infection for one year. As a result of this reasoning it was thought advisable to defer vaccination to the middle of August in order to obtain immune flocks during the months of September-December. The results did not confirm the assumption, as the unprotected birds developed fowl-pox during May. It became clear that fowl-pox outbreaks may and do occur in Palestine during the entire period of April-December inclusive and that in order to reduce its ravages birds had to be protected as soon as possible after hatching.

Vaccinating with pigeon-pox vaccine at least twice a year would most likely reduce the incidence of disease. However, many poultrymen object to this practice of repeated vaccination on the ground that either the vaccine or the handling of the birds affects egg production adversely.

It was apparent, therefore, that a vaccine which could be depended upon to give a complete immunity for at least one year was essential, and the object of the experiments reported below was to ascertain whether it is possible to retain the immunising value of fowl-pox vaccine and at the same time eliminate or reduce the risk of secondary lesions.

Experimental.

A series of preliminary experiments were conducted in the laboratory in which various combinations of pigeon-pox and fowl-pox vaccine and different sites of inoculation were used. The vaccines were prepared in a manner reported in a previous paper[1] and the following combinations were used :—

Experiment Ia.

(*a*) Pigeon-pox vaccine on one leg and fowl-pox vaccine applied with a brush on the other leg. Two birds ; takes on both legs ; one developed secondary lesions on the comb.

(*b*) Pigeon-pox and fowl-pox vaccine mixed and applied by brush on one leg. Two birds ; both had severe takes, characteristic of fowl-pox virus, with no secondary lesions.

(*c*) Pigeon-pox applied first, followed immediately on the same point with fowl-pox. Two birds ; both developed strong takes and secondary lesions and one died.

(*d*) Pigeon-pox on one leg and fowl-pox by stick on the wing ; nine birds. All developed takes on the leg ; eight had takes on the wing ; there were slight diphtheritic lesions in three.

(*e*) First pigeon-pox on the leg and two weeks later fowl-pox vaccine applied by the stick method on the wing. Eighty-three birds six months old were vaccinated. All had good takes with pigeon-pox ; all but four developed good takes on the wing ; eight of these had very strong takes on the wing. Three weeks after vaccination five birds developed mild secondary lesions on the comb.

Parallel with the first series of experiments another series was conducted with fowl-pox vaccine alone, using different methods and sites of inoculation.

Experiment Ib.

(*a*) Phenolised vaccine applied by brush on the leg ; eight birds. Seven developed good takes ; one developed secondary lesion on the eye and one a diphtheritic lesion.

(*b*) Phenolised vaccine applied by stick method on the wing ; six birds. All had good takes ; one developed a slight secondary lesion on the comb.

(*c*) Phenolised vaccine applied by stick method on the leg ; eight birds. Seven developed good takes ; one developed a secondary lesion.

Summarising these experiments it appears that (1) pigeon-pox vaccine did not give a complete immunity against the fowl-pox vaccine applied two weeks later ; but the number of birds showing secondary lesions was rather low, if the age of the birds is taken into consideration. (2) There was no advantage in using a double vaccine or a double method of vaccination.

Experiment II.—The effect of the mode of inoculation on the incidence of secondary lesions.

The object of these experiments was to determine the effect of the mode of inoculation and the locus on the appearance of

secondary lesions. These experiments were carried out at the Mikveh Agricultural School, and we are indebted to Mr. Fiat for his cordial co-operation. Incidentally the effect of chinosol as a water disinfectant was tested.

The experiments were carried out with 256 birds between four and five months of age, treated as follows :—

(*a*) One hundred fowls were vaccinated on the leg with phenolised fowl-pox vaccine by the brush method. Chinosol was put in the drinking water (1 gm. per 20 litres of water) as an antiseptic.

All the birds developed good takes ; some excessively large ; 37 birds had secondary lesions ; 17 pox lesions, 14 diphtheritic lesions and six lesions both on the head and in the mouth. The lesions healed and the birds recovered.

(*b*) Two groups of birds, one of 40 and the other of 38 birds, were vaccinated by the stick method on the wing. One group received chinosol in the water, the other did not. Of the 40 birds without chinosol seven developed secondary lesions, four had diphtheritic lesions and three pox lesions. Of the 38 with chinosol five developed secondary lesions, two had diphtheritic lesions, two had pox lesions, and one had both pox and diphtheritic lesions.

(*c*) A third group of 78 birds was vaccinated by the stick method on the leg. Of these birds 19 developed secondary lesions.

Three sets of untreated controls were kept ; ten in the first group, twelve with the fowls vaccinated by stick on the wing with chinosol in the drinking water, and nine with the group vaccinated by the stick method on the wing without chinosol in the drinking water. Of the ten controls in the first group four developed comb lesions ; of the twelve controls in the second group one developed lesions and of the nine controls in the third group two became affected.

Summarising these experiments it appears that the size and intensity of the primary take is an important source of secondary lesions. In the birds vaccinated by the stick method the takes are small and localised and the percentage incidence of secondary lesions is greatly diminished. Whereas with the brush method 37 out of 100 birds developed secondary lesions, the stick method on the leg gave 23·5 per cent. with secondary lesions, and with the stick method on the wing only 15 per cent. developed secondary lesions.

It seems, therefore, that the stick method reduces to a great extent the incidence of secondary lesions in vaccinated birds. These findings confirm those reported by Johnson.[3] It seems also that the wing is the most favourable site for vaccination.

Experiment III.—The effect of age on the incidence of secondary lesions.

The purpose of these experiments was to determine the effect of age on the incidence of secondary lesions.

Based on the previous experiments, the technique of vaccination and preparation of vaccine was standardised. The vaccine was prepared from fresh crusts of comb lesions of infected chickens. The material was removed, placed in Petri dishes and

dried in a dessicator over CaC_{12}. It was then ground thoroughly in a mortar, weighed and suspended in saline containing 50 per cent. glycerine, so as to give a 2 per cent. suspension. The vaccine was used in concentrated form. Although this vaccine retains its full effectiveness for at least one month, it was always used in the fresh state. The important elements in the technique were to employ only minute amounts of vaccine and to inoculate at a single point. In the following experiments the eye end of a small gauge sewing needle, as suggested by Beaudette,[4] was used. The inoculations were made in the centre of the wing by a quick piercing movement.

(*a*) On 26.3.35, 235 baby chicks four days old were vaccinated with fowl-pox vaccine by the stick method on the wing. On 4.4.35 they were examined for takes and all showed good takes. The takes increased in size and spread on both sides of the wing. On 17.4.35 the takes were still soft and spreading. The takes did not start to scab until 5.5.35.

Secondary lesions.—Secondary lesions were first noted on 17.4.35. Four chicks had diphtheritic lesions and two had pox lesions around the eye. The flock was depressed and appetite was impaired. During the next ten days the condition of the flock became worse and 23 additional affected chicks were noted. The mortality during the first month was 27 per cent. and the development was considerably retarded.

(*b*) On 19.2.35, 43 two month old pullets were vaccinated by the stick method on the wing. On 27.2.35 they were examined for takes and all showed good takes. The takes increased in size and attained about 1 cm. in diameter. The takes started to scab on 15.3.35.

Secondary lesions.—One vaccinated bird had a slight diphtheritic lesion. The flock remained in good condition.

(*c*) On 26.3.35, 48 pullets, two months old, were vaccinated by the stick method on the wing. All showed good takes, none had secondary lesions. The flock remained in good condition.

(*d*) On 21.3.35, 200 pullets, two and a half months old, were vaccinated by the stick method on the wing. All had good takes. One bird had a slight diphtheritic lesion, and one a pox lesion around the beak. Six birds affected with coccidiosis were destroyed.

(*e*) On 5.4.35, 1,140 pullets were divided into three groups in accordance with age and vaccinated by the stick method on the wing.

Group 1.—495 pullets, four months old ; eight had secondary lesions.

Group 2.—295 pullets, three and a half months old ; three with secondary lesions.

Group 3.—346 pullets, three months old ; three with secondary lesions.

Summarising these experiments, it is apparent (*a*) that it is dangerous to vaccinate baby chicks with fowl-pox vaccine ; (*b*) that healthy birds two to three months old may be vaccinated with fowl-pox vaccine without serious risk.

Experiment IV.—Comparative results of vaccination in early and late hatches.

The accompanying table gives the data on the various flocks vaccinated and the results. These data show that early hatched

birds, in good condition, can be successfully treated by the stick method on the wing with fowl-pox vaccine without any serious risk of secondary lesions or debilitating effects on the flock.

Late hatch birds gave less satisfactory results. It should be noted that birds which hatch in April or later develop very poorly. Coccidiosis is prevalent in these hatches and for this, as well as other unknown causes, probably climatic, the percentage of cull birds is rather high. It may be, therefore, that the less satisfactory results noted in these birds are ascribable to the generally depressed state of development of the birds. The following experiments are typical :—

(*a*) Two hundred pullets, two and a half months old, which hatched on April 4th, were vaccinated by the stick method, on the wing. Takes were good. No secondary lesions were noted and the flock developed as usual.

(*b*) 1,750 pullets were divided into three groups and vaccinated by the stick method on the wing.

Group 1.—500 pullets which hatched in the middle of April ; two months old.

Group 2.—450 pullets hatched at the end of March ; three months old.

Group 3.—800 birds which hatched the middle of February ; four and a half months old.

The takes were good in all groups. Sixteen birds with secondary lesions were noted in the third group ; eight with secondary lesions were noted in the second group, and two in the first group. Although the number of secondary lesions in the first group was the lowest, the condition of the flock after vaccination was the poorest. General weakness prevailed in the flock and many pullets had difficulty in moving about. The weakness lasted about one and a half months ; many pullets became emaciated and the percentage of culls was high.

(*c*) Three hundred pullets, two and a half months old, which hatched on 25.4.35, were vaccinated as above. The takes were good ; five birds showed secondary lesions. The flock became depressed on or about the third week after vaccination. The depression persisted for about a month. The percentage of culls was high.

(*d*) Three-hundred pullets which hatched the middle of April, three months old, were vaccinated as above. The takes were good. On the third week the appetite was impaired and the flock was very depressed. Twenty-three pullets died during the third week. The depression persisted and the flock did not pick up until much later.

Six more flocks of 2,754 chickens of middle or late April hatches were vaccinated. Although the number of birds showing secondary lesions was small, the post-vaccination reaction was severe, resulting in great emaciation and, in a few instances, in death.

Summarising the results of these experiments, it becomes obvious that fowl-pox vaccine cannot be recommended for use in flocks of late hatch.

Duration of Immunity.

Laboratory tests.—Twelve birds from different farms which had been vaccinated six months previously, together with six susceptible cockerels, were inoculated with fowl-pox virus at

different points on the comb, using virus dilutions of 1 : 1,000, 1 : 5,000, 1 : 10,000 and 1 : 40,000. The control cockerels were positive in all dilutions; the vaccinated birds were fully resistant.

Immunity to natural infection.—The following may serve as an example: In M.E., 800 birds were vaccinated with fowl-pox vaccine and 2,000 birds with pigeon-pox vaccine. The birds were kept in separate houses. Six-and-a-half months after the vaccination about 15 per cent. of the flocks vaccinated with pigeon-pox became infected with fowl-pox, while no cases were noted among those treated with fowl-pox.

Summary.

The laboratory and field experiments reported in this paper warrant the following conclusions :—

(1) Fowl-pox vaccine applied by the stick method on the wing to flocks two to three months old, of early hatch, results in few secondary lesions and confers a durable immunity.

(2) It is not advisable to use this vaccine in late hatched birds because these birds suffer from severe post-vaccination reactions.

It is suggested tentatively that late hatches be first vaccinated with pigeon-pox vaccine and two months later with fowl-pox.

TABLE I.

Records of Flocks Vaccinated with Fowl-pox Vaccine.

Name.	*No. of Pullets.*	*Age in Months.*	*Hatch : Date.*	*Secondary Lesions; No. of Birds.*	*Post-Vaccination Reaction.*
E.n.	52	2·2	Dec.–Jan.	1	None.
N.Z.	495	4	„	8	„
N.Z.	295	3·5	Jan.	3	„
N.Z.	346	3	Feb.	3	„
Ay.	200	2·5	Jan.	2	„
Sn.	3,850	2·3	Jan.–Feb.	15	None ; 32 birds killed because of debility.
Sn.	2,672	2·3	Feb.–March	21	None ; 21 birds killed during the first month because of debility.
S.B.	300	2·5	March	2	None.
Sn.	700	3	April	10	Severe post-vaccination reaction.
Yr.	2,000	2·5	„	0	Severe post-vac. reaction.
Sd.	300	3	„	5	„ „ „
At.	900	2·5	„	8	„ „ „

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